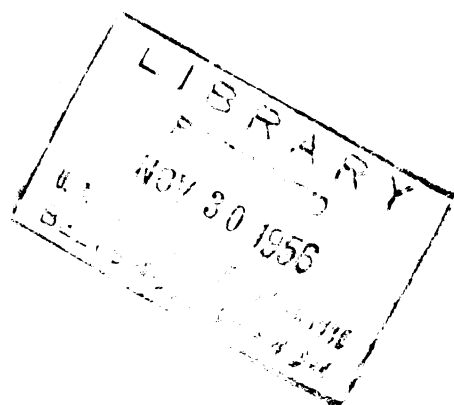


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PANTOTHENIC ACID



in foods

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PANTOTHENIC ACID in foods

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SUMMARY

A detailed laboratory procedure employing the double-enzyme system of intestinal phosphatase and pigeon-liver extract for release of pantothenic acid from foods and subsequent microbiological assay with *Lactobacillus plantarum* (formerly *Lactobacillus arabinosus*) is given along with results of its application. In a 2-year period this laboratory procedure, applied 104 times to a dried yeast sample, gave an average value of 111.0 µg. pantothenic acid/gm. with a standard deviation of 10.7 and a coefficient of variation of 9.7. Recoveries of pantothenic acid added to samples of dried beef, taken through the entire assay procedure, averaged 98.8 percent of the amount added and 99.9 percent of the total amount present. Reliability was also shown by good agreement between values obtained by this double-enzyme microbiological procedure and those obtained by rat bioassay on 5 widely different foods and yeast.

The results of the laboratory analyses of 208 food samples, representing 161 different foods using this method, are summarized (see table 8).

Analyses were always made on edible portions prepared from samples purchased in the retail market. In a few instances, processed foods were analyzed, but usually the assays were made only on raw products.

Lean muscle meat and poultry generally tended to be higher in pantothenic acid content than fruits and vegetables on a fresh-weight, edible-portion basis, although individual items in each class were found to overlap. Shell eggs, especially the yolks, were higher in pantothenic acid than meat. Most ready-to-eat cereals, whole-grain breads, and nuts (including peanuts) had about the same pantothenic acid content as meat. Fluid milk had about half the pantothenic acid found in meat.

BACKGROUND

Pantothenic acid, one of the B-vitamins, is of known nutritional importance because of its role in biochemical reactions as a part of coenzyme A, a mechanism only recently understood. Pantothenic acid has been demonstrated as a dietary

requirement for growth of a number of animals and different micro-organisms.

In 1933, R. J. Williams and coworkers (23)¹ reported an acid-yeast-growth factor and named the factor pantothenic acid. Seven years later the vitamin had been isolated, the chemical structure had been confirmed, and pantothenic acid was synthesized. Pantothenic acid is 2,4-dihydroxy-3,3-dimethylbutyryl-beta-alanide, and this dipeptide structure accounts for its instability to acid and alkali. Pantothenic acid has from the earliest studies been known to occur to some extent in all tissues or foods tested.

In early studies it was found that bioassays for pantothenic acid in natural materials employing rats or chicks generally gave higher results than microbiological assays on extracts of the materials treated with mylase-P, Takadiastase or papain (4, 5, 6, 10, 12, 18, 22). Microbiological assays using different test organisms also had been reported to give divergent results. These differences were subsequently found to be due to the utilization by the animals or microorganisms of a bound form or forms of the vitamin. Three bound forms of pantothenic acid have been found to occur naturally. While different forms of pantothenic acid serve as growth factors for various organisms, only coenzyme A serves as the biochemically active form of pantothenic acid in acetylation reactions.

In 1947 Lipmann and coworkers (11) reported that coenzyme A contained pantothenic acid, since established as 11 percent of the total coenzyme weight. Coenzyme A acts as an acetyl group transfer agent in tissue metabolism. This function helps to explain the mechanism whereby 2-carbon fragments resulting from fatty acid breakdown enter the cycle in which carbohydrate is oxidized to carbon dioxide and water, by way of pyruvic acid. Coenzyme A, through an active sulfhydryl group (—SH), unites with acetate and is the thiol ester shown to be "active acetate" which reacts with oxalacetate to form citric acid. The sulfhydryl group is part of the thioethylamine attached by peptide linkage to the alanine of pantothenic acid. Not only does coenzyme A take part in many acet-

¹ Italic numbers in parentheses refer to Literature Cited, p. 17.

ylations believed to be essential to the oxidative metabolism of carbohydrate, fatty acids, and amino acids, but also it has a role in steroid synthesis. Through coenzyme A, pantothenic acid becomes one of the essential activators for the reactions concerned with cellular oxidation of food materials. Since the pantothenic acid fraction of coenzyme A apparently cannot be manufactured by the human body but must be supplied in food, it is recognized as an essential nutrient.

Pantothenic acid occurs in foods in both free and bound forms. Release of the pantothenic acid molecule in coenzyme A was found to require enzymes other than those that had been used for liberating many vitamins from tissues. Novelli and coworkers (14) found pantothenic acid to be released quantitatively from coenzyme A only by a double-enzyme system involving intestinal phosphatase and pigeon- or chicken-liver extract.

Another bound form of pantothenic acid—the pantothenic acid conjugate (PAC) reported by King and coworkers (8, 9) as active for the growth of *Acetobacter suboxydans*—was found to contain phosphorus and glutamic acid. PAC apparently is not as easily dialyzable as coenzyme A, indicating a greater molecular weight than coenzyme A; and PAC apparently contains more glutamic acid on a molecular basis than coenzyme A. In the study, it was found that intestinal phosphatase and avian-liver enzymes liberate pantothenic acid quantitatively from PAC also.

Another form of bound pantothenic acid was reported by W. L. Williams and coworkers (24) to be required for the growth of certain strains of *Lactobacillus bulgaricus*. *L. bulgaricus* factor (LBF) was probably formed by the action of phosphatase on coenzyme A. It was similar to and possibly identical with the compound synthesized by Snell and coworkers (20), which was pantothenic acid joined by a peptide linkage to thioethylamine to yield

pantetheine. The double-enzyme system also splits this peptide linkage to give free pantothenic acid. LBF was an important compound in that it furnished a basis for establishing part of the probable structure of coenzyme A.

Colorimetric procedures (2, 16, 17, 21) for the determination of pantothenic acid in foods depend on reaction with a hydrolysis product of the vitamin, either beta-alanine (2, 16, 21) or pantolactone (17). They are restricted to relatively pure preparations of pantothenic acid in a fair concentration and have only limited application to extracts of foods and other natural materials (16).

Application of the double-enzyme system of Novelli and coworkers (14) to foods was made by Neilands and Strong (13). Thus, for the first time, microbiological assays on foods gave results equivalent to bioassay values. Novelli and Schmetz (15) improved the method, permitting the determination of pantothenic acid in low-potency material. With modification and standardization of the above procedures in the Human Nutrition Research Branch laboratories, Agricultural Research Service, United States Department of Agriculture, it became possible to obtain reliable data on the pantothenic acid content of a large number of foods.

The results reported here were on studies to standardize the extraction and microbiological procedures, to apply them systematically in the assay of common foods, and to report the quantitative distribution of total pantothenic acid among many typical foods which make up the American diet. Foods were also analyzed for their free pantothenic acid content, so that a comparison could be made with data found in literature reporting results obtained by older extraction methods which usually resulted in values only slightly higher than the free pantothenic acid content.

PART I. DEVELOPMENT OF MICROBIOLOGICAL PROCEDURE

STOCK SOLUTIONS FOR MEDIUM

Studies on the method for the microbiological assay for pantothenic acid and its comparison with rat bioassay have been reported earlier (22). The microbiological assay method is being given in sufficient detail in this handbook for direct use by the average laboratory worker. The conditions and times of storage of the following reagents, enzymes, and test organisms have been found to give reproducible standard curves and media free of contamination. These concentrations and amounts have been found convenient in this laboratory where the number of foods being assayed was large. Smaller amounts of stock solutions would probably be more practical in a laboratory where only a few analyses were contemplated.

The mention in this publication of a commercial company or of any commercial products does not imply endorsement by the United States Department of Agriculture over other companies or products not named.

Acid-hydrolyzed casein.—100 gm. of vitamin-free casein were mixed with 500 ml. 5 N HCl and refluxed 8 hours. The HCl was removed from the mixture by distillation under reduced pressure until a thick paste remained. The paste was dissolved in distilled water and concentrated again in the same manner. The resulting paste was redissolved in distilled water, adjusted to pH 3 (indicator paper) with concentrated NaOH, and sufficient water added to bring the volume to approximately 600 ml.; 40 gm. of Darco G-60 were added to the solution which was stirred 2 to 4 hours, and then filtered with suction through a pad of filter-cel.

The treatment was repeated using 10 gm. Darco for 1 hour if the filtrate was not colorless. Sufficient distilled water was added to bring the volume to 1 liter and the solution was stored under toluene at 5° C.

1 ml. \approx 100 mg. of hydrolyzed casein.

Tryptophan solution.—5 gm. of DL tryptophan were dissolved in 25 to 30 ml. of 1 N HCl, and sufficient water was added to make 250 ml. Solutions were stored up to 1 week under toluene at 5° C.

1 ml. \approx 10 mg. of L tryptophan.

Adenine, guanine, uracil solution.—200 mg. each of adenine sulfate, guanine hydrochloride, and uracil were dissolved with the aid of heat in 10 ml. of 20 percent HCl, and sufficient distilled water was added to make 1 liter. The solution was stored under toluene at 5° C.

1 ml. \approx 200 μ g. each of adenine sulfate, guanine hydrochloride, and uracil.

Xanthine solution.—400 mg. of xanthine were dissolved in 20 ml. of concentrated NH_4OH with heat, and distilled water was added to make 1 liter. The solution was stored under toluene at 5° C.

1 ml. \approx 400 μ g. of xanthine.

Vitamin-mixture solution.—40 mg. of riboflavin were suspended in 500 ml. of 0.02 M CH_3COOH , and the solution was protected from the light. 40 mg. of thiamine hydrochloride, 40 mg. of nicotinic acid, and 80 mg. of pyridoxine hydrochloride were dissolved in about 200 ml. of distilled water in a liter volumetric flask. 10 mg. of *p*-amino-benzoic acid were dissolved in distilled water and made to 100-ml. volume. 40 ml. of this *p*-amino-benzoic acid solution were pipetted into the liter volumetric flask; biotin to furnish 100 μ g. and the riboflavin solution were added quantitatively. Distilled water was added to volume, and the mixture was stored in a dark glass-stoppered bottle under toluene at 5° C.

1 ml. \approx $\left\{ \begin{array}{l} 40 \mu\text{g. each of riboflavin, thiamine} \\ \text{hydrochloride, and nicotinic acid;} \\ 80 \mu\text{g. of pyridoxine hydrochloride;} \\ 4 \mu\text{g. of } p\text{-amino-benzoic acid;} \\ 0.1 \mu\text{g. of biotin.} \end{array} \right.$

Salts solution A.—10 gm. of KH_2PO_4 and 10 gm. of K_2HPO_4 were dissolved in distilled water, diluted to 100 ml., and stored under toluene at room temperature. This solution was made fresh weekly.

1 ml. \approx 100 mg. each of KH_2PO_4 and K_2HPO_4 .

Salts solution B.—20 gm. of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 1 gm. of NaCl, 1 gm. of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, and 750 mg. of $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ were dissolved in distilled water; 1 ml. of concentrated HCl was added; the whole was diluted to 500 ml., and stored under toluene at room temperature.

1 ml. \approx $\left\{ \begin{array}{l} 40 \text{ mg. of } \text{MgSO}_4 \cdot 7\text{H}_2\text{O;} \\ 2 \text{ mg. each of NaCl and } \text{FeSO}_4 \cdot 7\text{H}_2\text{O;} \\ 1.5 \text{ mg. of } \text{MnSO}_4 \cdot \text{H}_2\text{O.} \end{array} \right.$

"Tween 80" solution.—2.5 gm. of polyoxyethylene sorbitan monooleate² were dissolved in warm

distilled water (45° C.), diluted to 500 ml., and stored under toluene at 5° C.

1 ml. \approx 5 mg. of polyoxyethylene sorbitan monooleate.

Table 1 lists the ingredients used in the basal medium and gives the concentration in 2 ways—the amount of each ingredient per tube, and the amount of each solution or weight of solid for 1 liter of double-strength medium, as prepared and used in these investigations.

ASSEMBLING THE MEDIUM

Distilled water to approximately half of the expected volume was placed in the container for the medium, and the liquids were added without warming, in the order shown (table 1). The solids were then added and dissolved with a minimum of stirring. The pH was adjusted to 6.8 with HCl or NaOH, with the use of a pH meter. The medium was made to volume, layered with toluene, and returned to the refrigerator until time to put it into the assay tubes.

TABLE 1.—Composition of basal medium used for assay of pantothenic acid with *L. plantarum*

Reagent	Concentration of solid per assay tube	Amount for 1 liter of double-strength medium
Acid-hydrolyzed casein.	50 mg-----	100 ml.
DL tryptophan-----	2 mg-----	20 ml.
Adenine, guanine, uracil.	50 μ g. each adenine, guanine, and uracil.	50 ml.
Xanthine-----	100 μ g-----	50 ml.
Vitamin mixture-----	20 μ g. pyridoxine; 10 μ g. each thiamine, riboflavin, niacin; 1 μ g. paba; 0.025 μ g. biotin.	50 ml.
Salts A-----	5 mg. each KH_2PO_4 and K_2HPO_4 .	10 ml.
Salts B-----	2 mg. $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$; 100 μ g. each NaCl and $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$; 75 μ g. $\text{MnSO}_4 \cdot \text{H}_2\text{O}$.	10 ml.
"Tween 80"-----	500 μ g-----	20 ml.
Dextrose, anhydrous--	200 mg-----	40 gm.
Sodium acetate $\cdot 3\text{H}_2\text{O}$ --	83 mg-----	16.6 gm.
L cystine ¹ -----	1 mg-----	200 mg.

¹ Dissolve in about 5 ml. of 10 percent HCl before adding to the medium.

OTHER REAGENTS

The following reagents are needed for either the microbiological-assay procedure or the extraction of the pantothenic acid from the foods.

Physiological salt solution.—9 gm. of NaCl were dissolved in water and diluted to 1 liter. Sterile salt tubes were prepared by placing 10 ml. portions in test tubes, plugging with absorbent cotton, and autoclaving for 15 minutes at 15 pounds of steam pressure.

² "Tween 80," Atlas Powder Co., Wilmington, Delaware.

Bromthymol blue indicator saturated solution.—1 gm. of bromthymol blue was suspended in 16.5 ml. of 0.1 N NaOH, and the volume was brought to 250 ml. with distilled water. This was discarded after standing if the solution was not still green.

Solubilized liver extract.—5 gm. of Bacto liver were suspended in 100 ml. of distilled water. The mixture was held at 50° C. for 1 hour and at 80° C. for 5 minutes, filtered, and the filtrate stored under toluene in a glass-stoppered bottle at 5° C.

"Tris" buffer—1 M, pH 8.3.—24.2 gm. 2-amino-2- (hydroxymethyl) -1,3-propanediol $[(\text{CH}_2\text{OH})_3\text{CNH}_2]^3$ were dissolved in distilled water and diluted to 200 ml., the pH adjusted with a pH meter to 8.3 with 30 percent HCl, the solution filtered through Whatman No. 40 filter paper into a glass-stoppered bottle, and stored at room temperature. This solution was discarded when more than 2 weeks old.

"Tris" buffer—1 M, pH 11.3.—24.2 gm. 2-amino-2- (hydroxymethyl) -1,3-propanediol were dissolved in distilled water, diluted to 200 ml., filtered, and stored in a glass-stoppered bottle at room temperature.

Sodium bicarbonate buffer—0.1 M, pH 8.5.—850 mg. NaHCO_3 were dissolved in distilled water and made to 100-ml. volume. This solution was prepared fresh daily.

REAGENTS FOR ENZYME PREPARATION

The following reagents were used in the preparation of enzymes for this determination.

Potassium bicarbonate—0.02 M.—1 gm. KHCO_3 was dissolved in ice-cold distilled water, diluted to 500 ml., and stored in the refrigerator until used. This solution was prepared fresh each time it was used.

Activated Dowex.—100 gm. of Dowex-1 (200–400 mesh) were stirred by means of a glass stirrer with sufficient power to keep the Dowex completely suspended, in a liter of 1 N HCl for 10 minutes, then filtered through Whatman No. 50 filter paper on a Buchner funnel by means of suction. The residue was treated in the same manner with a second liter of N HCl for another 10 minutes, and then filtered. The residue was washed with a liter of distilled water, being stirred and filtered under identical conditions. The washings were repeated 10 times. The acid-treated washed Dowex was wetted sufficiently with water to mix well, and "tris" buffer pH 8.3 was added drop by drop until the pH was 8.0. The slurry was stored in a refrigerator for use within about 2 days.

ENZYME PREPARATION

Intestinal-phosphatase solution—2 percent.—A water extract of the alkaline intestinal-phosphatase preparation (Armour and Company) was prepared fresh daily, 5 ml. being allowed for each 4 ml. to be used.

³ P-4833—Eastman Chemical Products, Inc.—tris (hydroxymethyl) aminomethane.

Pigeon-liver extract—10 percent.—All equipment used, including centrifuge tubes and cups, mortar and pestle, and pipettes, were cooled overnight in the freezing compartment of a home type refrigerator. As the acetone-dried pigeon liver received from Armour had a high percentage of feathers and connective tissue, it was sifted through a 50-mesh Monel-metal sieve to concentrate the liver. After being sifted, the liver was weighed into 10-gm. lots, placed in small bottles, and returned to the freezer. For the extraction, 10 gm. were rubbed to a paste with about half of the 100 ml. ice-cold 0.02 M KHCO_3 in a mortar and pestle held in an ice-salt bath. The suspension was transferred as quantitatively as possible to ice-cold centrifuge tubes with the assistance of the remainder of the KHCO_3 .

The contents of each centrifuge tube were thoroughly stirred with a small glass rod, placed in balanced centrifuge cups, and chilled in the freezer compartment for 10 minutes. Centrifugation at 3,000 revolutions per minute (r. p. m.) for 5 minutes was followed by a cooling period of 10 minutes in the freezer. The supernatant fluid was transferred to a cold 125-ml. Erlenmeyer flask, about half the 100 grams of activated Dowex were poured in, and the flask was shaken for 5 minutes in the ice-salt bath.

The mixture was then transferred to balanced cold centrifuge tubes, centrifuged for 5 minutes at 3,000 r. p. m., and returned to the freezer for a 10-minute chilling.

The supernatant fluid was treated again in the same manner with the remainder of the activated Dowex; and after being chilled, it was poured into cold centrifuge tubes and centrifuged for 5 minutes, then cooled for 10 minutes. The supernatant fluid was then transferred to balanced cold centrifuge tubes, centrifuged at 3,000 r. p. m. for 5 minutes, returned to the freezer for a 10-minute chill, and then pipetted into small, sterile, cold tubes in amounts ranging from 2 to 5 ml., depending upon the expected need. The small tubes were then frozen in the refrigerator and thawed just prior to use.

TEST ORGANISM—ITS CARE AND INOCULUM PREPARATION

The following reagents were used in maintaining the culture.

Test organism.—*Lactobacillus plantarum* (formerly *Lactobacillus arabinosus* 17-5), culture No. 8014, obtained from the American Type Culture Collection, 2029 M Street NW., Washington 6, D. C., was used for these assays.

Agar culture medium.—5 gm. Bacto yeast extract, 5 gm. dextrose, and 7.5 gm. agar, were made up to 500 ml. with distilled water. The agar was dissolved by steaming for 10 minutes. The hot solution was tubed in 10-ml. amounts; the tubes were then plugged with cotton and autoclaved 15 minutes at 15 pounds pressure.

If refrigerated, the sterile-agar tubes were usable for about 6 months; if kept at room temperature, about 2 months.

Liquid culture medium.—Broth-culture-medium tubes were prepared by placing 5 ml. basal medium (see table 1), 5 ml. distilled water, and 1 drop of solubilized liver extract into lipless test tubes. These tubes were plugged with cotton and autoclaved 15 minutes at 15 pounds pressure. The sterile-broth tubes were kept at room temperature, and fresh tubes were prepared every 2 to 3 weeks.

Stock culture of *L. plantarum*.—The pure culture of *L. plantarum* was maintained by monthly transfers from agar through broth tubes for 24 hours and back to agar. The broth tubes were incubated in a water bath held at 34° C. for 24 hours, and agar tubes inoculated from these rapidly growing cultures were incubated for 18 to 24 hours, and stored in the refrigerator. At biweekly intervals the inoculum was started from these agar tubes.

Broth inoculum.—Cells of *L. plantarum* to be used for inoculum were maintained by daily transfer from broth to broth. Cells were never used for inoculum unless they had been transferred twice through broth. For use as inoculum, the cells were separated from the broth by centrifuging for 15 minutes at 2,500 r. p. m.; the liquid was then decanted, and the cells were resuspended in sterile saline and centrifuged for 10 minutes; the wash was repeated once, and the cells were resuspended in a third portion of sterile saline.

STANDARD PANTOTHENIC ACID SOLUTIONS AND STANDARD SAMPLE

Stock A.—Approximately 45 mg. of reference-standard calcium pantothenate,⁴ dried under vacuum over P₂O₅, were weighed on a microbalance, transferred to a liter volumetric flask, and dissolved in about 500 ml. distilled water. 100 ml. 0.2 N sodium acetate and 10 ml. 0.2 N acetic acid were added, and the whole was brought to volume with distilled water. Using the factor—

$$1.087 \mu\text{g. calcium pantothenate} = 1.000 \mu\text{g. pantothenic acid,}$$

the concentration of pantothenic acid per ml. was determined. This solution was stored in the refrigerator, and was prepared fresh each year.

Stock B.—4- $\mu\text{g. pantothenic acid/ml.}$ —A portion of stock A pantothenic acid solution was taken to give 1 liter of a solution containing *exactly* 4.0 $\mu\text{g. pantothenic acid per ml.}$ To this portion, 90 ml. 0.2 N sodium acetate and 9 ml. 0.2 N acetic acid were added, and the whole was diluted to volume with distilled water. This was prepared every 3 months and was also kept refrigerated. Working standards were prepared from this stock.

Working standards.—The 3 working-standard dilutions were prepared fresh for each assay. For the 0.1- $\mu\text{g-per-ml. pantothenic acid standard,}$ 5 ml. stock B (4.0- $\mu\text{g. pantothenic acid per ml.}$) was diluted to 200 ml. with distilled water. For the 0.02- $\mu\text{g-per-ml. pantothenic acid standard,}$ a second 5-ml. portion of stock B pantothenic acid standard was diluted to 1,000 ml. with distilled water. The 0.01- $\mu\text{g. pantothenic acid per ml. standard}$ was prepared by diluting 25 ml. of the 0.1 $\mu\text{g. standard}$ to 250 ml. with distilled water.

Standard reference sample.—A quantity of moist brewer's yeast⁵ was prepared for a standard sample in the laboratories of the Human Nutrition Research Branch. The wet yeast was separated by filtration with suction on a large Buchner funnel, dried in a vacuum desiccator over CaCl₂, ground through a 60-mesh screen with a Wiley mill, and stored in a glass bottle in the refrigerator.

EXTRACTION OF PANTOTHENIC ACID FROM FOODS

Sample extractions.—The edible portion of most food samples was divided into 6 representative parts, sealed in No. 2 sanitary enameled cans, and stored until used at -40° F. For analysis, the cans of meats, poultry, nuts, cheese, fresh fruits, and vegetables, and also the dried fruits, were opened as soon as they were removed from the freezer. The entire contents of each can of frozen food was put through a home electric food chopper with a plate having openings of $\frac{1}{16}$ -inch diameter, or $\frac{1}{32}$ -inch if impossible to use the finer plate. From 4 to 100 gm. of the finely ground, unthawed food was weighed immediately on a small torsion balance, and transferred quantitatively to a Waring Blendor cup containing 10 ml. "tris" buffer pH 8.3. Sufficient distilled water was added to obtain a good suspension while the total volume was kept below 150 ml. About 1.5 ml. of caprylic alcohol was added, to prevent foaming in the Blendor and bumping in the autoclave.

The whole was blended for 3 to 5 minutes and then transferred quantitatively, with the aid of a funnel and an 8-inch swab stick, to a 200-ml. volumetric flask, the volume being kept below 190 ml. The flasks were plugged with cotton, autoclaved at 15 pounds pressure for 15 minutes, and cooled to room temperature. The samples were then brought to volume and shaken vigorously. For many samples, a second Blendor treatment lasting about 1 minute was necessary at this time, to obtain a suspension that could be pipetted accurately.

An entire subsample of cereals, grains, whole-wheat flour, or dried vegetables, was put through a 60-mesh screen in an intermediate model Wiley mill. Amounts of 10 to 25 gm. of this finely divided sample were then treated in exactly the same way as the moist samples.

⁴ Obtained from U. S. P. Reference Standards, 46 Park Ave., New York 16, N. Y.

⁵ Obtained from Christian Heurich Brewing Co., Washington, D. C.

Breads and certain dried foods had to be handled by a slight modification of the above procedure. For these foods, the finely ground samples were put directly into 500-ml. Erlenmeyer flasks marked at the 200-ml. volume. "Tris" buffer pH 8.3 and caprylic alcohol were added, and the whole was brought to volume. The flasks were shaken as thoroughly as possible by hand, plugged with cotton, and autoclaved as usual. After being cooled to room temperature, the volume was adjusted if necessary, and the entire contents of the flask were transferred to a Waring Blendor and blended from 3 to 5 minutes.

Free pantothenic acid.—To determine the free pantothenic acid value, 3 different-sized aliquots of the food extracts were treated (omitting enzymes) under conditions identical with those described in the following paragraphs. Because the free pantothenic acid was always less than the total, the aliquots taken for determination were at least twice those used for enzyme treatment and, in some instances, considerably more.

Total pantothenic acid.—To determine the total pantothenic acid content, a double-enzyme treatment was given to 3 different-sized aliquots of each food. Test tubes measuring 16 mm. in diameter were used for these extractions and the total volume in each tube treated with enzymes was kept at 2.1 ml. A 0.1 ml. of 0.1 M sodium bicarbonate buffer, together with sufficient distilled water to bring the total volume to 2.1 ml. (allowing for the sample aliquot and the 0.6 ml. of the enzyme system), were pipetted into each tube.

Sample weights taken for enzyme digestion varied from 8 to 700 mg. of the fresh foods, whose moisture content varied from 65 to 96 percent. For the dry foods, whose moistures ranged from 2 to 12 percent, the samples used for the enzyme treatment were from 20 to 175 mg.

Aliquots of the food extracts were pipetted with care to keep the sample from the sides of the tube, and the tubes for total pantothenic acid determination were placed in an ice bath. To each tube, 0.4 ml. 2 percent intestinal phosphatase and 0.2 ml. twice-Dowex-treated 10 percent pigeon-liver extract were added. Each tube was thoroughly mixed by hand shaking—care being used to keep the sample off the sides of the tube—then layered with toluene, plugged with cotton, and incubated overnight (or 4 hours if more convenient) in a water bath held at 37° C.

Standard sample and blank.—A standard sample of brewer's yeast—aliquoted at 0.8, 1.2, and 1.6 ml. for free pantothenic acid content, and at 0.4, 0.6, and 0.8 ml. for total pantothenic acid content—was run with every assay. A blank determination was also run with each assay; and because the pigeon liver is responsible for most of the pantothenic acid found in the blank, it was added in 1, 1½, and 2 times the amount used with the samples.

Dilution and filtration.—After incubation, the contents of the tubes were transferred quantita-

tively to 100-ml. volumetric flasks, made to volume, and filtered through dry fluted Whatman No. 40 filter paper. With certain foods low in pantothenic acid, where increased sample weight could not be handled, it was necessary to limit the final volume to 50 ml. Refiltering through the same filter paper helped to clarify most samples and was routinely done.

MICROBIOLOGICAL PROCEDURE

Assay procedure.—Pyrex 16-mm. tubes with plastic screwcaps were used for the microbiological assay. The filtrates were used directly in amounts of exactly 1, 2, 3, 4, and 5 ml. in triplicate assay tubes. A standard curve, also in triplicate, was run for each of 15 levels at 0.0, 0.0, 0.005, 0.010, 0.015, 0.020, 0.025, 0.030, 0.040, 0.050, 0.060, 0.080, 0.100, 0.100, and 0.200 µg. pantothenic acid per tube, respectively. The first 0.0 tubes were not inoculated and were used as a check on sterility of the medium and tubes. A different working standard was used in the second set of 0.100-µg. tubes, thus giving a check on the dilution of the standard.

Routinely in advance of assay, the tubes were numbered, and water was added in sufficient amounts to give a total volume of 5 ml. per tube after the standard or sample was added. These watered tubes were then stored in a walk-in refrigerator held at 32° F. The laboratory temperature was kept around 70° F. in the winter and was air-conditioned at 80° F. in the summer. The filtrates and standards were kept at room temperature until used unless 3 hours or more were expected to elapse. Under these conditions, the extracts and standards were refrigerated until a short time before use. The assay tubes were refrigerated at 5° C. after the samples or standards were added.

When all extracts, blanks, and other solutions had been pipetted, each tube received 5 ml. of the cold double-strength basal medium (see table 1) by means of an automatic pipetting machine. Each tube was capped, and the entire set was autoclaved for 10 minutes at 15 pounds pressure. (If a pipetting machine is not used, the contents of the tubes must be shaken before they are autoclaved.) After being cooled to room temperature, all tubes except the first triplicate 0.0 standard tubes were inoculated aseptically with 1 drop of the washed *L. plantarum* cells, by use of the apparatus shown (fig. 1). After inoculation, the tubes were incubated in water baths held at 34° C. for 72 hours. At the end of the incubation period, the contents of each tube were titrated with 0.1 N NaOH, 2 drops of the bromthymol blue indicator being used per tube.

Titration.—Tubes were titrated with a titration apparatus (fig. 2) designed in a laboratory of the Human Nutrition Research Branch. Essentially, the apparatus is a titration stand with a built-in fluorescent light to which 2 burettes with 2-way stopcocks are mounted so that they drain into 2

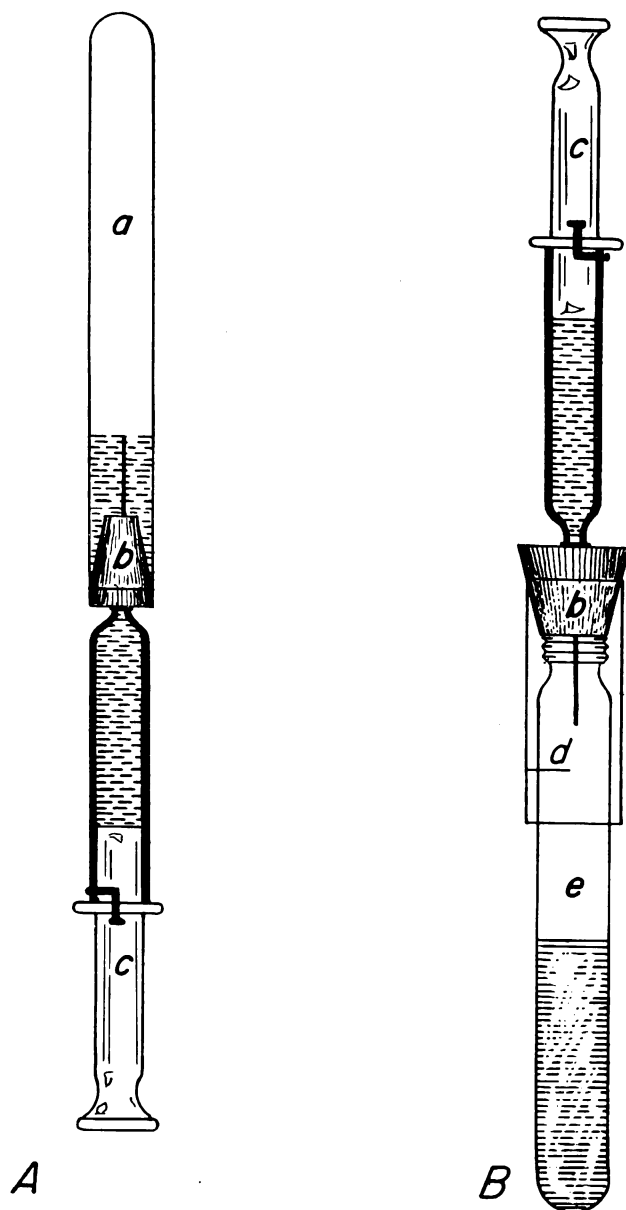


FIGURE 1.—Apparatus used for aseptic inoculation of microbiological-assay tubes: A, Transferring inoculum to sterile syringe; B, tube in position for drop of inoculum; a, straight test tube (16- by 150-mm.) in which inoculum was grown and washed; b, 22-gage needle embedded in rubber stopper; c, 5-ml. syringe with spring holder; d, glass guard (22- by 70-mm.); e, screwcap assay tube in position for drop of inoculum.

pyrex separatory funnels (used as titration vessels) connected to a filter pump (water-operated) for emptying the titrated samples and the rinse water down the drain. The level of alkali in the burettes is automatically maintained by another airejector (filter pump). The samples are added to the titration flasks via tubing connected to a funnel which also has a built-in rinsing system. Compressed air is used to agitate the samples during the titrations. The stopcocks contain no grease and, with practice, 1,000 tubes could be titrated in an 8-hour working day.

Calculation.—The concentrations in $\mu\text{g.}$ of standard pantothenic acid per tube versus the average ml. of standard alkali used in titrations were plotted on "log-log" paper. The type of standard curve obtained with these data is shown (fig. 3).

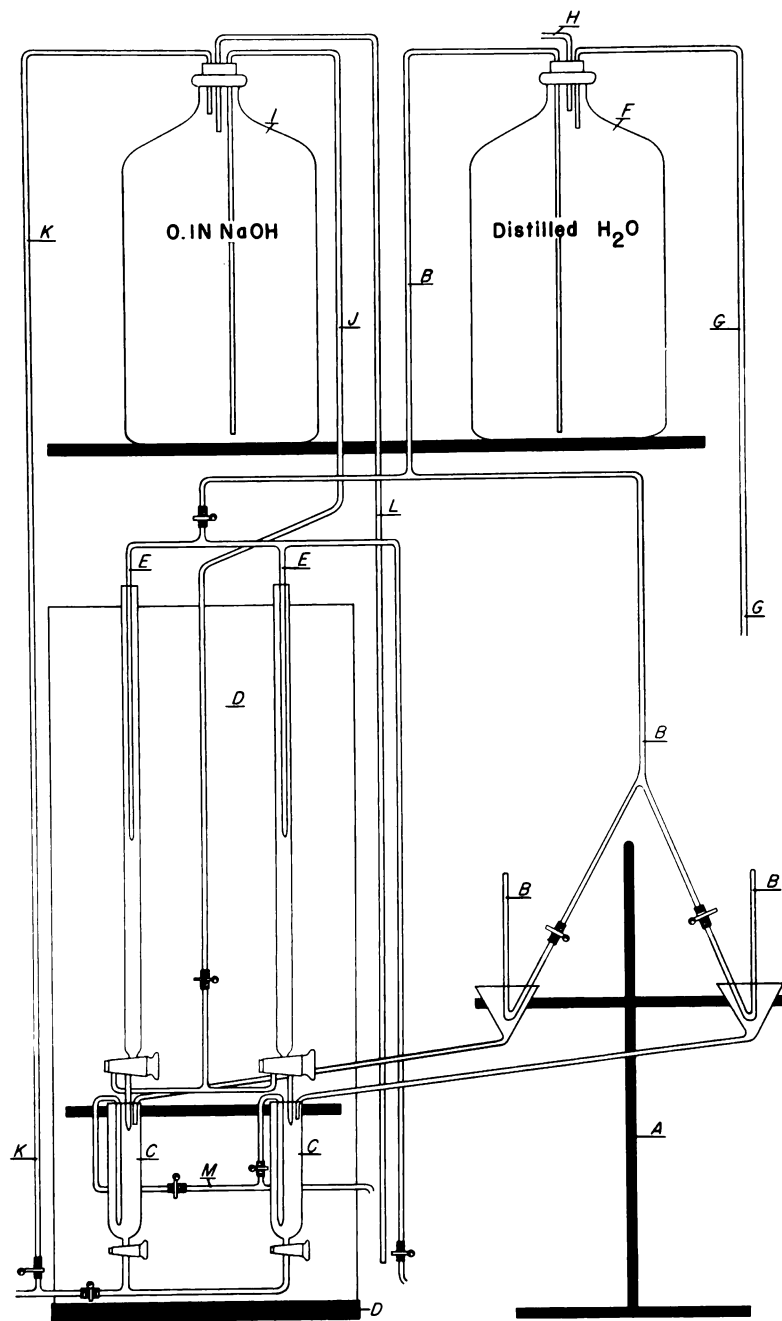
By using the standard curve of the assay period and the titrations and dilutions of the sample, the free and total pantothenic acid for each food were calculated, taking into account the blank determination obtained with the enzymes for the total pantothenic acid calculation. For every food, 3 subsamples were analyzed in at least 2, and in most cases 3, assay periods. Fresh extracts from replicate subsamples were prepared when repeat assays for any food were needed.

INVESTIGATIONS TO ESTABLISH THE METHOD

The first experiments performed to develop, standardize, and evaluate the microbiological procedure just described for determining free and total pantothenic acid in foods are reported by Toepfer and coworkers (22). The results of these investigations established: (1) The amounts of intestinal phosphatase and pigeon-liver enzyme preparation used in the extraction procedure; (2) the fact that mylase-P released only 9 to 60 percent of the bound pantothenic acid released by intestinal phosphatase-liver enzymes in the 10 foods studied; (3) reproducibility with a 5-fold variation in sample size for extraction and a 10-fold change in sample-aliquot size for enzyme incubation; and (4) the fact that bioassays with rats were in satisfactory agreement with the microbiological assays. Further experimental work undertaken during the course of the study, and the results obtained with the microbiological assay in another laboratory, as well as in this study, are given later in this publication.

Basal medium.—In the beginning of the study, several media and organisms were tested for response to increments of pantothenic acid. The optimum response was obtained with the Skeggs and Wright (19) medium with *L. arabinosus* (now *L. plantarum*). Further experimentation was limited to this medium with this organism. In checking additions to the medium, a growth response was obtained with an increase of xanthine; the amount of xanthine was therefore doubled over the original concentration.

Considerable attention was given to obtaining a basal medium not stimulated by fat, since fat has been reported (6, 19) and also observed in this laboratory to stimulate *L. plantarum* (formerly *L. arabinosus*) growth. The addition of 100 mg. "Tween 80" per liter of medium was found satisfactory for samples to which known fatty acids were added. To check further on the adequacy of this medium in the assay of foods, 6 foods varying from 1.7 to 51.4 percent fat and from 1.4 to 76.6 percent moisture were chosen,



A.—Stand holding 2 funnels, each used for introduction of sample to its titration flask.

B.—Distilled-water-rinse system used to rinse contents of tubes into titration flasks and also to rinse titrated samples after titration.

C.—Pyrex separatory flasks used as titration vessels, connected to airejector (filter pump) for emptying titrated samples and rinses.

D.—Titration stand with fluorescent light, holding two 50-ml. burettes with 2-way stopcocks and a rack for holding titration vessels.

E.—Glass tubing with a fine tip connected by a T-tube and clamps to either a second airejector, for automatic zeroing of the standard alkali in the burettes, or to the distilled-water reservoir for rinsing the burettes after use.

F.—Distilled-water reservoir with 3-hole rubber stopper.

G.—Distilled-water inlet.

H.—Overflow tube leading to the sink.

I.—Standard alkali reservoir with 3-hole rubber stopper.

J.—Connection to burettes with a clamp shutoff.

K.—Glass tubing with a clamp shutoff connected to the airejector for vacuum to use in filling the alkali reservoir.

L.—Connection to alkali reservoir, used for filling.

M.—Glass tubing with a capillary ending connected to a compressed-air source for agitation of the samples during titration.

FIGURE 2.—Apparatus used for semiautomatic titration of microbiological-assay tubes.

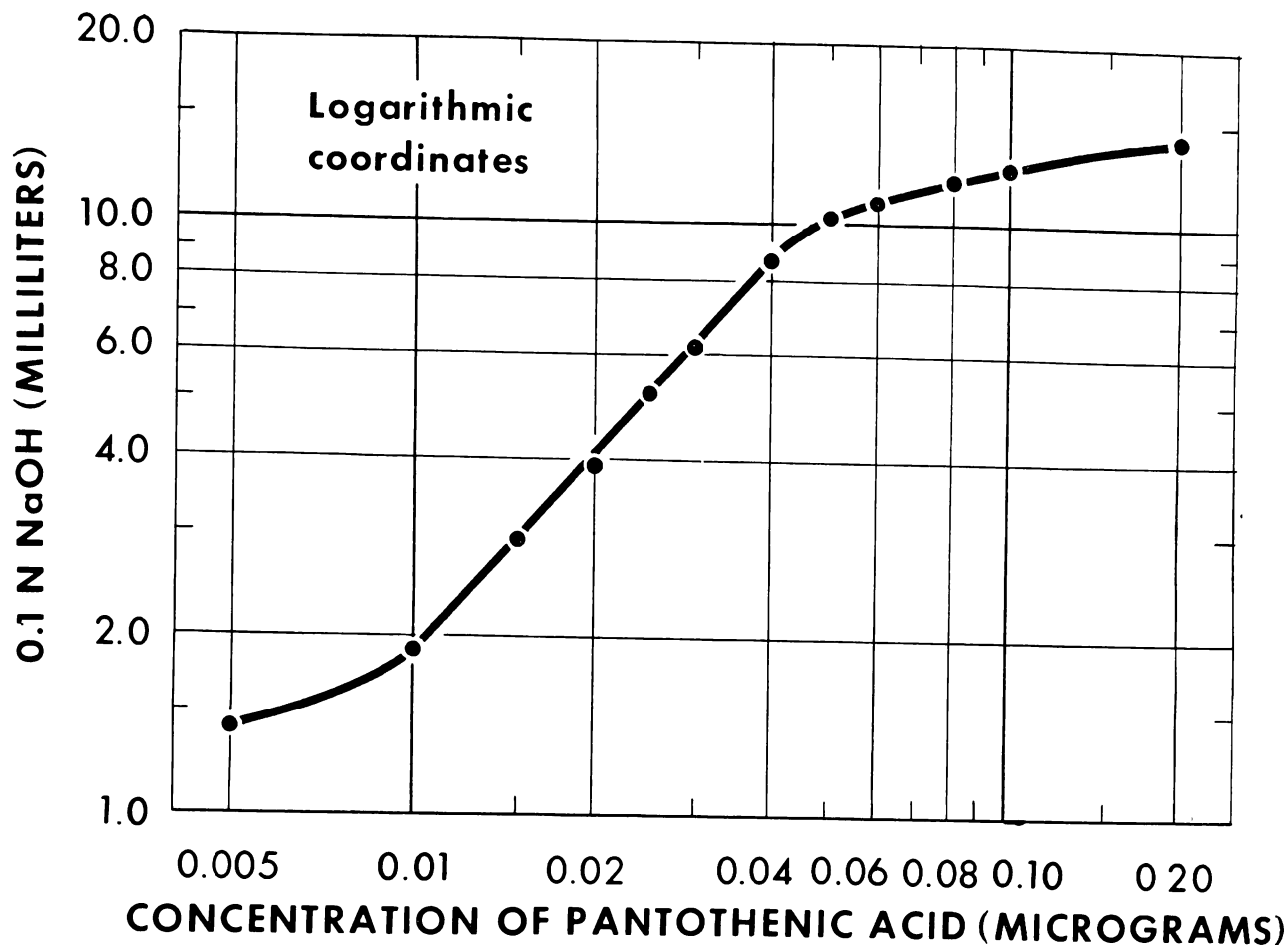


FIGURE 3.—Acid production of *L. plantarum* in response to increments of pantothenic acid.

and pantothenic acid assayed on both the residue after ethyl ether extraction and the fresh food. The finely ground fresh foods were packed tightly into small Whatman extraction thimbles, placed in a Soxhlet apparatus, and subjected to continuous ethyl ether extraction for 24 to 30 hours. The samples were then transferred to beakers, broken up with a spatula, and dried in a desiccator over

CaCl₂ until used. For assay, a weighed quantity of this fat-free dry residue was treated exactly as a regular food sample.

The data thus obtained are presented (table 2). In general, the agreement between the adjusted values obtained from the moisture, fat, and pantothenic acid determinations and the values obtained on the ether-extracted sample are good.

TABLE 2.—Pantothenic acid values, reported on a dry, fat-free basis, found in ethyl ether extracted and nonextracted food samples with and without double-enzyme treatment

Food	Fat (ether extract)	Moisture	Free pantothenic acid		Total pantothenic acid	
			Ethyl ether extracted	Adjusted from fresh weight	Ethyl ether extracted	Adjusted from fresh weight
	Percent	Percent	μg./gm.	μg./gm.	μg./gm.	μg./gm.
Beef, ground round	12.8	65.9	13.68	13.80	24.30	30.80
Cheese, cheddar	34.5	33.3	6.17	7.55	12.23	12.58
Chicken, breast	1.7	76.2	27.61	30.81	39.06	37.92
Chicken, legs	3.7	76.6	31.87	42.54	50.35	59.80
Chicken, skin	24.5	60.9	29.44	32.88	46.10	47.40
Peanuts	51.4	1.4	32.52	34.68	43.80	51.14

pH of the extracting solution.—"Tris" buffer first reported by Gomori (3) had been used by Novelli and Schmetz (15) with excellent success in the enzyme treatment of samples for microbiological assay. Since its buffering capacity was known to be effective for large amounts of HCl, it was chosen for the food extractions. With fresh fruits such as lemons and limes, where the pantothenic acid content was low, the food was very acid, and large samples had to be taken (100-gm.). There were indications that the 10-ml. "tris" buffer used in the standard procedure were insufficient to neutralize the food sample. An experiment with limejuice of pH 2.3 was run, in which various amounts of "tris" buffer pH 11.3 were used. The lime aliquots incubated for this experiment were 1.2, 1.5, and 1.75 gm. per tube without enzyme; and 300, 400, and 500 mg. per tube with enzyme. This 100-gm. limejuice sample required 89 ml. of the "tris" buffer pH 11.3 to bring the pH to 6.6. The results of this experiment and of a series of standards run over the same pH range (table 3) show considerable pantothenic acid destruction below pH 6.0. The destruction was greater in the limejuice sample.

Because of these results, 14 of the more acid foods were checked. Lemons, limes, cranberries, rhubarb, and sour red cherries were too acid for the standard procedure. These were run by the modified procedure to give values for the table of pantothenic acid content of foods.

TABLE 3.—*Influence of pH on the destruction of pantothenic acid during sample extraction*

pH	Pantothenic acid found		
	0.0140 µg./ml. standard solution	Limejuice	
		Free	Total
	µg./ml.	µg./gm.	µg./gm.
2.3-----		0.36	0.19
2.5-----	0.0111		.28
3.5-----			.34
4.5-----	.0124		.91
5.5-----	.0129		1.29
6.0-----	.0133		
6.6-----	.0137	.65	1.45
7.0-----	.0137		
7.2-----			1.41
7.5-----	.0135		
8.0-----	.0137		
11.0-----	.0102		

Recovery of added pantothenic acid.—Three levels of standard pantothenic acid were added directly to duplicate 6-gram samples of dried ground beef round before extraction, so that the recovery figures would be representative of the entire procedure. The data (table 4) show the recoveries

calculated in 2 ways: First, as total recovery, which ranged from 94.5 to 104.7 percent, with an overall average of 99.9 percent; and second, as added pantothenic acid recovery, which ranged from 85.7 to 108.5 percent, with an average of 98.8 percent.

TABLE 4.—*Recovery of pantothenic acid added to samples of air-dried ground beef round*¹

Standard pantothenic acid added	Calculated total pantothenic acid	Pantothenic acid found	Pantothenic acid recovered	
			Total	Added
	µg./gm.	µg./gm.	Percent	Percent
13.83 µg./gm.-----	36.03	{ 35.07 34.05	97.3 94.5	93.1 85.7
20.66 µg./gm.-----	42.86	{ 42.22 44.45	98.5 103.7	96.9 107.7
27.67 µg./gm.-----	49.87	{ 50.18 52.22	100.6 104.7	101.1 108.5
Average-----	-----	-----	99.9	98.8

¹ Pantothenic acid content of samples was 22.20 µg./gm.

Reproducibility of assays.—To check further the reproducibility and reliability of the microbiological-assay procedure, 5 foods and yeast were also assayed by the microbiological procedure at the Texas Agricultural Experiment Station. The data obtained in both laboratories, together with the 95-percent confidence limits calculated from the standard error of the average, are given (table 5).

Rat-bioassay comparison.—To be certain that the enzyme system chosen was adequate, rat bioassays were also conducted on the same 5 foods and yeast. As a further check on the medium using high-fat foods, 2 high-fat foods (peanuts and dried whole egg) were included. The foods chosen and the results of the rat bioassays and of the microbiological assays, together with the 95-percent confidence limits, are given (table 6). These are the bioassays reported by Toepfer and coworkers (22) along with the detailed procedures used in conducting the bioassays. The results by microbiological assay agreed with the results of the rat bioassay with the exception of carrots. The results of the microbiological assays on the 2 high-fat foods—peanuts with 50.8 percent fat, and dried whole egg with 41.4 percent fat—fall well within the 95-percent limits of the bioassays of those foods.

Taking agreement with rat bioassays as the criterion, the microbiological method used for this study gave very satisfactory values for total pantothenic acid as utilized by mammals.

TABLE 5.—*Pantothenic acid values obtained by 2 laboratories by assay with L. plantarum in 5 foods and yeast, with and without the double-enzyme treatment*

Food	Free pantothenic acid				Total pantothenic acid			
	Laboratory No. 1		Laboratory No. 2		Laboratory No. 1		Laboratory No. 2	
	Average	95-percent limits	Average	95-percent limits	Average	95-percent limits	Average	95-percent limits
	$\mu\text{g./gm.}$	$\mu\text{g./gm.}$	$\mu\text{g./gm.}$	$\mu\text{g./gm.}$	$\mu\text{g./gm.}$	$\mu\text{g./gm.}$	$\mu\text{g./gm.}$	$\mu\text{g./gm.}$
Carrots.....	14.2	14.6–13.8	17.5	19.9–15.0	20.7	21.9–19.5	23.1	26.6–19.5
Dried whole egg.....	50.7	55.0–46.4	57.6	61.7–53.5	76.2	80.1–72.3	66.4	72.7–60.0
Kale.....	41.4	44.0–38.8	44.1	45.8–42.4	64.9	68.4–61.4	59.4	66.6–52.2
Peanuts.....	12.4	13.3–11.5	12.0	12.9–11.1	23.8	25.5–22.1	17.7	21.4–14.0
Liver, pork.....	96.0	98.9–93.1	85.7	101.6–69.7	203.0	213.7–192.3	151.6	166.2–137.0
Yeast, brewer's.....	46.3	47.4–45.2	54.8	64.2–45.3	117.6	122.1–113.1	117.7	140.4–94.9

TABLE 6.—*Pantothenic acid values obtained by rat bioassay and by assay with L. plantarum using the double-enzyme treatment*

Food	Bioassay (rat)			Microbiological assay	
	Number of animals	Average	95-percent limits	Average	95-percent limits
		$\mu\text{g./gm.}$	$\mu\text{g./gm.}$	$\mu\text{g./gm.}$	$\mu\text{g./gm.}$
Carrots.....	60	35.7	51.4–24.9	¹ 20.7	21.9–19.5
Dried whole egg.....	60	61.5	83.0–45.6	76.2	80.1–72.3
Kale.....	² 48	56.2	81.7–38.6	64.9	68.4–61.4
Peanuts.....	60	25.4	33.6–19.3	23.8	25.5–22.1
Liver, pork.....	60	176.7	244.7–127.5	203.0	213.7–192.3
Yeast, brewer's.....	48	138.0	169.0–112.7	117.6	122.1–113.1

¹ Significant difference from bioassay mean.

² High-level supplement not eaten; therefore only low-level-supplement animals used.

RESULTS WITH THE ESTABLISHED PROCEDURE

Using this standardized procedure, the free and total pantothenic acid contents were determined on meats, poultry, eggs, nuts, fresh and dried fruits and vegetables, breads, breakfast cereals, rice, flour, milk and cheese.

Sample titrations.—To illustrate the agreement among aliquots in response to increasing amounts of sample, the titrations obtained for each tube in 4 samples for both free and total pantothenic acid are plotted (fig. 4). To make it possible to follow the points for each sample more easily, a smoothed curve was drawn for each, but in actual calculation the pantothenic acid content of each aliquot of a food was determined individually. All the titration values for the 3 different aliquots of each food are given.

These 4 foods, taken as examples of laboratory data from 4 different assay periods, illustrate directly the range of sample sizes used and indirectly the pantothenic acid content found during the assay of foods.

As an example of liver, one of the foods high in pantothenic acid, rabbit liver averaging 45.03 $\mu\text{g./gm.}$ was the first food presented. The original laboratory sample taken for analyses weighed 5 gm., and the 3 aliquots used for enzyme treatment represented, respectively, 15, 20, and 25 mg. of liver.

As an example of lean-muscle meat, a good source of pantothenic acid, leg of lamb averaging 8.86 $\mu\text{g./gm.}$ was the second food illustrated. The laboratory sample taken for analysis weighed 32 gm., and the 3 aliquots taken for enzyme treatment represented, respectively, 96, 120, and 160 mg. of the lean meat.

As representative of fresh vegetables, carrots assaying 2.72 $\mu\text{g./gm.}$ was the third food chosen. The laboratory sample weighed 60 gm., and aliquots taken for enzyme treatment were 180, 240, and 300 mg., respectively.

The fourth food, green seedless grapes with 0.40 $\mu\text{g./gm.}$, was the lowest in pantothenic acid content of the common foods analyzed. The laboratory sample weighed 100 gm., and the 3 aliquots taken for enzyme treatment were 500,

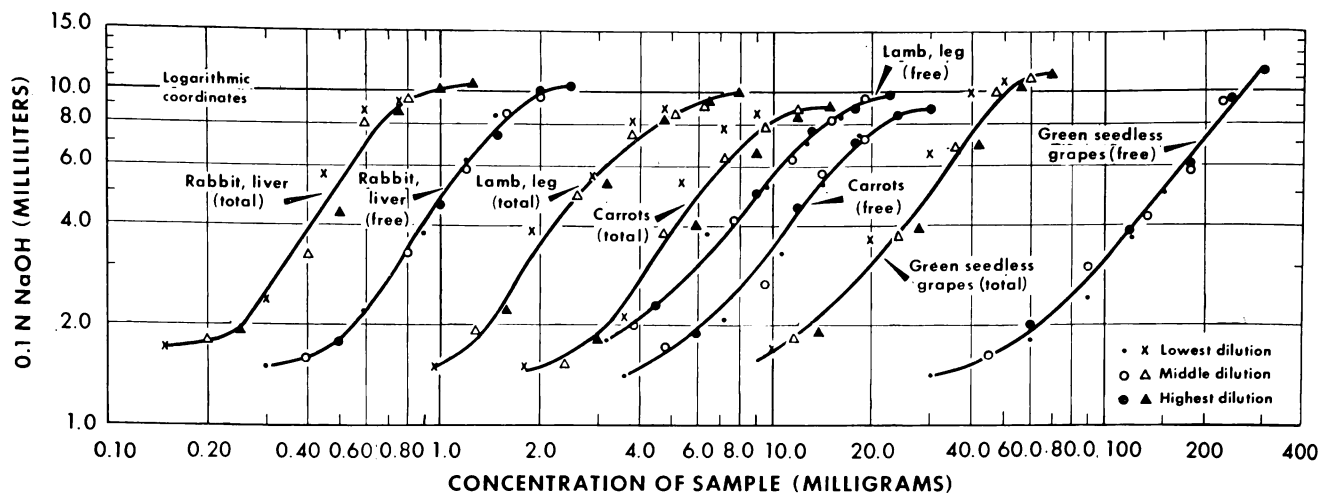


FIGURE 4.—Titrations obtained in different foods with and without double-enzyme treatment, as measured by *L. plantarum*.

600, and 700 mg., respectively. The grape aliquots were diluted after incubation to 50 ml., in order to be in a good range on the standard curve.

The agreement between the 3 aliquots, as judged by the titrations shown, was excellent. In all examples, at least 3 increments of sample in each aliquot were in agreement; in many, all 5. In the medium used, *L. plantarum* also responded to increments of samples, showing excellent agreement with the response obtained to standard pantothenic acid.

Effectiveness of the Dowex treatment of pigeon liver.—During the 2-year period of this study, the average amount of pantothenic acid remaining in the liver extract after 2 Dowex treatments was 0.0023 μg . Since the standard curve is best from 0.01 to 0.100 μg ., a blank of this magnitude could be handled.

At one point in the study, a metal stirrer (stainless-steel) was substituted for the glass stirrer in the Dowex activation. It was found that the pigeon liver treated with this Dowex lost almost 20 percent of its activity, as judged by the release of pantothenic acid from the standard brewer's yeast. Differences between values obtained in different laboratories are probably due to differences in the enzyme preparation.

Standard reference sample.—The brewer's yeast was analyzed 104 times during this study, exclusive of the experimental runs. The average value for the free pantothenic acid was 44.04 $\mu\text{g./gm.}$, with 95-percent and 99-percent confidence limits of 44.73 to 43.35 and 44.96 to 43.12, respectively. For total pantothenic acid content, the average value was 110.6 $\mu\text{g./gm.}$, with 95- and 99-percent confidence limits of 113.05 to 108.87 and 113.73 to 108.19, respectively.

Comparison with literature values.—Since Novelli and coworkers published the results of their

research in 1949 (14), 49 of the foods reported in this handbook were analyzed in other laboratories by the double-enzyme system. These data have been assembled (table 7) along with 3 rat and 24 chick bioassay values, as well as the microbiological-assay data obtained in the Human Nutrition Research Branch laboratories on these foods. If several values were given in the published findings, an average value was used on the table, and the number of reported values used to obtain the average are indicated. Of the 4 microbiological-assay values obtained with the double-enzyme system before Dowex treatment of the pigeon liver for removal of the pantothenic acid in the liver (13), 3 are considerably higher than any obtained in the present study, and are in line with the results obtained in this laboratory with untreated pigeon liver.

The 3 rat-bioassay values were reported from 2 different laboratories; and 2 of these foods—walnuts and wheat bran—have chick-bioassay values in addition. The walnut values obtained by rat- and chick-bioassay were in fair agreement, and the microbiological-assay value fell between them. The rat bioassay value for wheat bran was double the chick-bioassay value. The microbiological-assay value in this study checked a value found in literature, and both fell nearer the chick-bioassay value. All the chick bioassays were conducted in 1 laboratory and represent several years of work, including certain assays conducted before crystalline pantothenic acid was synthesized. With 5 foods, the chick-bioassay values were higher than the microbiological-assay values obtained in this study.

Since this study was initiated, Schweigert and Guthneck (18) have published a method for the determination of pantothenic acid in meats, in which Dowex-treated hog-kidney extract is used with excellent results instead of the pigeon-liver extract. These values are included in table 7.

TABLE 7.—*Pantothenic acid values reported in the literature, determined by microbiological assay using the double-enzyme system, and by bioassay with rats and chicks in comparison with values found in this study*

Food	Free pantothenic acid		Total pantothenic acid				Literature cited
	Microbiological assay in		Microbiological assay in		Bioassay in literature		
	Literature	This study ¹	Literature ¹	This study ¹	Rat ¹	Chick ¹	
MEATS							
Beef:	<i>μg./gm.</i>	<i>μg./gm.</i>	<i>μg./gm.</i>	<i>μg./gm.</i>	<i>μg./gm.</i>	<i>μg./gm.</i>	
Kidney		26. 57	34. 10 (2)	40. 64			(18)
Liver		60. 57	68. 38 (4)	93. 36			(18)
Rib		2. 02	4. 1 (5)	5. 42		10	(18), (7)
Dried	7. 9	8. 70	² 47. 5	23. 40			(13)
Lamb:							
Kidney		36. 41	43. 3	47. 43			(18)
Liver		44. 08	81. 4	70. 99			(18)
Leg		2. 89	5. 9	8. 86			(18)
Pork:							
Liver		30. 01	66. 98 (2)	70. 08			(18)
Ham, precooked		3. 82	5. 5	6. 94			(18)
Rabbit:							
Liver	1. 2	28. 75	75	45. 03			(14)
Muscle	5. 1	5. 20	9. 9	7. 90			(14)
EGGS							
Whites		. 79(4)	2. 87	1. 44(4)			(1)
Yolk		36. 08(4)	31. 21	42. 35(4)		63 (4)	(1), (7)
Whole	10. 2	13. 14(4)	² 52. 6	15. 76(4)		27 (37)	(13), (7)
NUTS							
Almonds		2. 81	4. 09	5. 78			(1)
Cashews		5. 50	14. 88	11. 62			(1)
Coconuts:							
Meat		. 84	2. 07	1. 93			(1)
Milk		. 35	. 0052	. 50			(1)
Walnuts		5. 91		9. 70	10. 9	8	(5), (7)
VEGETABLES, FRESH							
Beans, green snap		. 38	2. 14	2. 02			(1)
Broccoli		7. 74		12. 87		13 (2)	(7)
Cabbage		1. 56(3)	1. 63	2. 80(3)			(1)
Carrots		1. 50	3. 15	2. 72		2	(1), (7)
Corn, yellow		3. 06		8. 89		8 (3)	(7)
Eggplant		. 90	2. 29	2. 05			(1)
Kale		7. 25		12. 88		3 (2)	(7)
Lettuce		1. 19	1. 36 (2)	3. 63			(1)
Onion		. 52	1. 27	1. 68		1. 2(2)	(1), (7)
Peppers, green		. 71	2. 29 (3)	2. 37			(1)
Potatoes, white		2. 87(3)	2. 67	4. 05(3)		6. 5(2)	(1), (7)
Spinach	7. 5	1. 36	² 25. 7	3. 12		1. 2(2)	(13), (7)
Squash, zucchini		1. 67	3. 06	3. 40		3	(1), (7)
Sweetpotatoes		8. 16	5. 33 (4)	9. 35		11 (2)	(1), (7)
Tomatoes		. 69	. 92	3. 14		1 (2)	(1), (7)
VEGETABLES, DRIED							
Beans:							
Cowpeas		7. 68	9. 22 (4)	12. 43		18 (2)	(1), (7)
Kidney		2. 53	5. 12	6. 50			(1)
Lima		6. 01	7. 98 (4)	12. 99			(1)
Navy		4. 15	4. 98 (2)	12. 08			(1)
Lentils		9. 80	11. 44	14. 99			(1)
Split peas		12. 79(2)		21. 16(2)		21 (2)	(7)

See footnotes at end of table.

TABLE 7.—*Pantothenic acid values reported in the literature, determined by microbiological assay using the double-enzyme system, and by bioassay with rats and chicks in comparison with values found in this study—Continued*

Food	Free pantothenic acid		Total pantothenic acid				Literature cited
	Microbiological assay in		Microbiological assay in		Bioassay in literature		
	Literature	This study ¹	Literature ¹	This study ¹	Rat ¹	Chick ¹	
FRUITS	<i>μg./gm.</i>	<i>μg./gm.</i>	<i>μg./gm.</i>	<i>μg./gm.</i>	<i>μg./gm.</i>	<i>μg./gm.</i>	
Avocados	-----	9. 27	10. 90	11. 35	-----	-----	(1)
Bananas	-----	1. 84	2. 09 (3)	3. 06	-----	. 7(2)	(1), (7)
Figs, dried	-----	2. 91	-----	4. 03	7. 0	-----	(4)
Lemons	-----	1. 89	1. 08	2. 65	-----	-----	(1)
Lime, juice	-----	. 65	3. 14	1. 38	-----	-----	(1)
Oranges	-----	1. 26(2)	1. 89 (2)	2. 21(2)	-----	. 7(2)	(1), (7)
Pineapple	-----	. 75	1. 47	1. 75	-----	-----	(1)
CEREALS, OTHER GRAIN PRODUCTS							
Oats, rolled	-----	5. 03	10. 35	14. 13	-----	11	(1), (7)
Cornmeal	-----	2. 58(2)	4. 31 (2)	5. 93(2)	-----	-----	(1)
Rice, white	-----	4. 46	5. 21 (2)	6. 40	-----	4 (2)	(1), (7)
Wheat	-----	6. 34(2)	12. 06 (16)	10. 95(2)	-----	11 (2)	(16), (7)
Wheat bran	23. 0	19. 40	² 30. 0	29. 03	48 (2)	24 (4)	(13), (10), (7)
Yeast, brewers	-----	26. 04(2)	124. 56 (2)	99. 24(2)	-----	-----	(1)
MILK, CHEESE							
Milk:							
Skim	-----	2. 78(2)	-----	3. 75(2)	-----	3. 6(9)	(7)
Whole	-----	2. 34(6)	3. 43	3. 23(6)	-----	2. 8(4)	(1), (7)
Cheese, cottage	-----	1. 15	1. 98	2. 80	-----	-----	(1)

¹ Number in parentheses is number of microbiological or animal assays averaged to obtain figure.

² Data obtained before Dowex treatment of pigeon-liver enzyme.

PART II. QUANTITATIVE DISTRIBUTION OF PANTOTHENIC ACID IN TYPICAL FOODS

FOOD SAMPLES

Meat.—Meats were purchased during the winter months, to avoid transportation and preparation during Washington, D. C., summers. Hamburger, ground beef, stew meat, liver, ham slices, and other samples were purchased from at least 3 retail markets in the city, and pooled. For pork loins, veal legs, and similar cuts, care was taken to make certain that the cut was from at least 3 different animals and, usually, that more than 1 market was represented. For packaged meats, such as frankfurters, sausage, and liverwurst, 1 package of each brand found in the stores on the day of purchase was obtained and combined.

Laboratory-sample preparation involved obtaining the edible yield from each item and dividing the lean from the separable fat. The laboratory sample used for analysis was prepared by combining the edible lean from each item on an equal-

weight basis, the weight of the smallest item being used as the combining unit. On the packaged meats, all brands were cut to the smallest unit of weight, and the whole combined for analysis. Six subsamples were made of each of the combined samples of food purchased; these subsamples were sealed in No. 2 sanitary enameled tin cans and stored at -40° F.

Poultry.—For poultry, legs and breasts were purchased separately for the chicken samples, and the right halves of 3 birds were procured for the turkey samples. All the edible meat was used for analysis; the dark and white muscle meat, organs, and skin were analyzed separately. Subsamples were prepared and stored, as for meats.

Eggs.—Four lots of eggs, each lot consisting of 2 dozen assorted-sized brown and white eggs, were obtained from Animal and Poultry Husbandry Research Branch, Agricultural Research Service,

in June, July, and October. The analyses were started immediately. No samples were stored, and yolks and whites were analyzed separately.

Nuts.—Nuts, with the exception of cashews, were purchased in the shell. The peanuts were purchased roasted, the others as they are sold for home consumption. The nuts were shelled in the laboratory, yields obtained, and subsamples stored, as were meats.

Fresh vegetables and fruits.—Fresh vegetables and fruits were obtained on the Washington, D. C., market at the peak season whenever feasible. State of origin and variety, if known, were also recorded. Each market lot purchased was analyzed separately, and usually only one lot was obtained. Lots of foods arrived in the laboratory in the late afternoon and were stored overnight in a large walk-in refrigerator held near 35° F. The foods were washed in distilled water, if necessary, and dried back to the original weight by blotting with cheesecloth. Edible portions were prepared and yields calculated for all vegetables and fruits. If more than one part of a vegetable or fruit was edible and the structures were very different, the parts were analyzed separately. Six 200-gm. subsamples of each vegetable and fruit were prepared and stored, as for meats. Potatoes and apples were not frozen; a preliminary assay was run as soon as the food was received in the laboratory; the remainder of the sample was stored in the walk-in refrigerator; and final analyses were run as soon as the results of the first analysis were completed. Analyses were run separately on the peeled apples and potatoes and also on the peel.

Dried vegetables and fruits.—One package of each brand of a single type of bean, pea, or fruit easily available on the Washington, D. C., market was purchased. The various brands of each item were combined in equal weights, the edible weight of the smallest unit being used as the combining weight. Subsamples of approximately 50 gm. of dried vegetables and 100 gm. of dried fruits were canned and stored at -40° F.

Breads.—One loaf of sliced bread of each type baked by the main commercial bakeries of the Washington, D. C., area was purchased on the open market. Breads from 1 to 9 bakeries were included in each of the 8 types of bread analyzed. For laboratory samples, each loaf of each type of bread was cubed; an equal weight of cubes from each bakery was combined; the whole was thoroughly mixed; and subsamples were withdrawn, packed in cans, and stored at -40° F.

Breakfast cereals.—One package of each breakfast cereal usually found in the large markets of the Washington, D. C., area was purchased. These packages were then grouped first according to kind of grain, and second according to preparation of the grain. Products from 1 to 5 food processors were included in each of the 13 cereal composites analyzed. Equal weights from each manufacturer were combined for the laboratory samples. Six 50-gm. subsamples were stored at -40° F. for the laboratory analyses.

Flour.—With the exception of white enriched all-purpose flour which came from three milling companies, the flours were a single brand obtained on the Washington, D. C., market.

Grains.—One sample of the 1952 spring-wheat crop was obtained from the Grain Division of the Agricultural Marketing Service. The other grains were purchased on the Washington, D. C., market. Each sample analyzed was a composite of products obtained from 1 to 5 processors. The composites were made on an equal-weight basis from each processor; and 6 subsamples, each 50-gm., were canned and stored at -40° F. until analyzed.

Milk.—The buttermilk and the evaporated milk were obtained on the Washington, D. C., market from 4 dairies and 3 milk-processing companies, respectively. The nonfat dry-milk solids were from 2 market purchases, 1 in each year of the study. The first year's purchase was from the 1952 process, and the laboratory sample included the product of 3 food processors. The second purchase was from the 1953 process, and the laboratory sample included the product of 4 food processors. Two samples of raw whole milk and one each of heavy cream, pasteurized whole milk, skim milk, and homogenized milk were obtained from the Dairy Husbandry Research Branch of the Agricultural Research Service. One sample each of pasteurized whole, skim, and homogenized milk were obtained from one dairy in the Washington area. Each of these fluid milk samples was separately analyzed immediately upon receipt at the laboratory. Evaporated-milk samples were stored in a home type refrigerator and were opened and composited at the time of analysis. Dried milk composites were stored at -40° F. until analyzed.

Cheese.—All the cheese samples were obtained from the Washington, D. C., market. The cheddar was from the 1951 process from both New York and Wisconsin. The cottage cheese was a composite of 5 brands carried in the large markets of the Washington area. The processed-cheese sample was American type cheese, composited from products made by 7 processing companies. All laboratory composites were made from equal weights of each brand, and the subsamples were canned and stored at -40° F. until analyzed.

MOISTURE

Moisture was determined on weighed 1- to 10-gm. samples of food, depending on the expected moisture content. Samples were dried to constant weight in a vacuum oven at 70° C., in aluminum-foil moisture dishes.

FAT

Fat was extracted from weighed, dried samples under continuous extraction with ethyl ether in a Soxhlet apparatus for 18 hours. After the ether was evaporated, the fat was dried for 5 days in a vacuum desiccator over fresh calcium chloride and then weighed.

DETAILED DATA OBTAINED IN THE STUDY OF 237 FOOD ITEMS

A large summary of free and total pantothenic acid in the edible portion of foods, containing data on 237 food items, is given (see table 8). All the foods were analyzed between November 1952 and December 1954. A total of 1,139 assays in 109 assay periods, consisting of 509 subsamples for the free and 630 subsamples for the total pantothenic acid data, form the basis of the report. Of these items, 29 are values calculated to include the fat trim of meats as well as the separable lean, which was analyzed. For certain vegetables and fruits, the yield data on different portions, such as roots and leaves, were used to calculate a composite value for the total edible material. The pantothenic acid content of whole fresh eggs was obtained from the proportions of white and yolk which were analyzed separately.

With most foods, the free pantothenic acid was about half the total pantothenic acid. For each food item, the yield figure is shown which represents the portion used for the laboratory sample and from which the subsamples were prepared. The reported moisture figure and percentage of fat, or ether extract, are averages of duplicates on each subsample of food tested.

Analyses were made of 25 meat items. The separable lean of beef averaged 0.661 mg./100 gm.; that of pork, lamb, and rabbit, 0.788 mg./100 gm.; and that of veal averaged the highest, with 1.063 mg./100 gm. fresh weight. It is interesting to note that the muscle of the young, rapidly growing animal was the highest in pantothenic acid content. The overall separable lean muscle from all species analyzed was 0.883 mg./100 gm. fresh weight and 2.632 mg./100 gm. dry weight. As expected, organ meats of all animals were high in pantothenic acid content. The fresh kidney of large animals (beef, veal, and lamb) averaged 4.273 mg., with a range of 4.011 to 4.743 mg./100 gm. In general, liver had the highest pantothenic acid content of the fresh foods analyzed, with beef, lamb, pork, veal, and rabbit averaging 7.531 mg./100 gm. Beef and veal were the highest of the livers with an average of 9.522 mg./100 gm., while pork, lamb, and chicken liver averaged 7.090 mg., with a range of only 7.008 to 7.163 mg./100 gm., and rabbit liver was lowest with 4.503 mg./100 gm. fresh weight.

In both chicken and turkey, the dark meat was higher in pantothenic acid than the white. The dark meat of both averaged 1.153 mg./100 gm. fresh weight, while the white meat averaged 0.715 mg./100 gm. fresh weight. Generally the chicken meat contained more pantothenic acid than the turkey.

On a fresh-weight basis, the 4 lots of egg yolks from the Agricultural Research Center, with an average of 4.235 mg./100 gm., contained 30 times the amount found in the egg whites, which averaged 0.144 mg./100 gm. On a dry-weight basis, how-

ever, the yolks with 8.241 mg./100 gm. contained 7 times the amount in whites, with 1.229 mg./100 gm. Whole eggs were a good source of the vitamin, averaging 1.576 mg./100 gm. fresh weight. Over 80 percent of the pantothenic acid found in eggs was in the free form.

Eight varieties of tree nuts were analyzed. On a fresh-weight basis, coconuts were the lowest in pantothenic acid, with 0.193 mg./100 gm., and pecans were the highest with 1.707 mg./100 gm. On a dry-weight basis, Brazil nuts were lowest, with 0.241 mg./100 gm., while pecans were still highest, with 1.751 mg./100 gm. The average of all the common tree nuts was 0.808 mg./100 gm. fresh weight.

Three market lots of roasted peanuts were analyzed and an average pantothenic acid content of 2.137 mg./100 gm., with a range of 1.651 to 2.414 mg., was found.

The 5 varieties of dried beans averaged 1.009 mg./100 gm. of pantothenic acid; lentils contained 1.499 mg./100 gm.; and dried peas contained 2.116 mg./100 gm. Full-fat soy flour, which was the only form of soybeans analyzed, contained 1.681 mg./100 gm. Corn cereal with soya added contained considerably more pantothenic acid than plain-corn cereals.

No relationship could be found between pantothenic acid content and protein content of the tree nuts, peanuts, and dried legumes. In these foods, the protein content varied from 36.54 to 2.34 gm./100 gm., while the pantothenic acid content of the same foods varied from 2.414 to 0.193 mg./100 gm. in a highly irregular fashion. In 4 of these foods, the protein content varied only from 24.11 to 24.33 gm. while the pantothenic acid varied from 0.650 to 2.182 mg./100 gm. in no definite order.

Fresh vegetables are not a particularly concentrated source of pantothenic acid. However, in the amounts usually consumed, they would contribute a considerable amount of this vitamin to the diet. Mushrooms were the highest source of the vitamin among vegetables, having 2.713 mg./100 gm. Other important fresh-vegetable sources were broccoli, cauliflower, kale, and sweet potatoes. Other fresh vegetables having more than average amounts of pantothenic acid were sweet corn, peas, brussels sprouts, parsnips, and asparagus. Green onion bulbs, with 0.168 mg./100 gm., had the lowest pantothenic acid content of any common vegetable analyzed. On a fresh-weight basis, peeled potatoes of 3 varieties averaged 0.404 mg./100 gm., while the whole potatoes averaged 0.380 mg. It is interesting to note that the pantothenic acid content of the paring (0.230 mg./100 gm.) is about half that of the potato.

Of the foods analyzed, fresh fruits were the lowest in pantothenic acid. Avocado, with 1.135 mg./100 gm., was the fruit analyzed with highest content; and green seedless grapes, with 0.040 mg./100 gm., the lowest. Fresh fruits with about 0.3 mg./100 gm. were bananas, watermelon, strawberries, and apricots.

Raisins, like grapes, were very low in pantothenic acid. However, the other commercially dried fruits analyzed averaged 0.575 mg./100 gm. Dates contained the greatest amount, 0.780 mg./100 gm.

Grain products followed a pattern, the highest in pantothenic acid being the least refined. Whole-wheat bread with 0.789 mg./100 gm. was highest, and vienna bread with 0.378 mg./100 gm. was lowest. Breakfast cereals averaged 0.863 mg./100 gm. Wheat bran assayed 2.903 mg./100 gm., oatmeal 1.413 mg./100 gm., and corn cereals 0.239 mg./100 gm. Buckwheat and whole-wheat flour ranked with breakfast cereals as sources of the vitamin.

Bearing out the observation on refinement, the amount of pantothenic acid found in enriched all-purpose flour was approximately 50 percent of the amount found in whole-wheat flour. White rice contained only 62 percent of the vitamin found in brown rice. Precooked rice was still lower, with less than 50 percent of the vitamin content of the white rice on a dry-weight basis. These findings indicate that the cooking and drying processes leached or destroyed some of the pantothenic acid.

Fluid whole milk averaged 0.323 mg./100 gm. Fresh fluid skim milk and reconstituted dry skim milk averaged 0.399 mg./100 gm. with a range of 0.362 to 0.426 mg./100 gm. The fresh skim milk tested was a little lower than the reconstituted nonfat solids, indicating little or no loss in processing.

Of the cheese samples analyzed, cottage was lowest in pantothenic acid content with 0.280 mg./100 gm.; cheddar contained 0.405 mg./100 gm.; and processed American type was highest, with 0.482 mg./100 gm. When cheese on a dry-weight, fat-free basis is compared with milk on the same basis, only 40 percent of the pantothenic acid is accounted for in the cheese, indicating a loss during processing.

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TABLE 8.—*Pantothenic acid in edible portion of foods, as determined by microbiological assay with L. plantarum with and without double-enzyme extraction*

Food	Yield of edible portion (raw or processed)	History of sample, including month in which obtained, source, ¹ and composition	Free pantothenic acid					Total pantothenic acid				
			Fat (ether extract)	Moisture	Assay periods	Fresh-weight basis		Sub-samples	Assay periods	Fresh-weight basis		Dry-weight basis
						Range	Average			Range	Average	
	Percent	Percent	Percent	Percent	Num-ber	mg./100 gm.	mg./100 gm.	Num-ber	Num-ber	mg./100 gm.	mg./100 gm.	mg./100 gm.
MEATS												
Beef:												
Hamburger.....	100	63.3	16.8	63.3	2	0.288-0.320	0.314	3	2	0.660-0.693	0.681	3.422
Kidney.....	88	77.7	3.0	77.7	2	2.606-2.707	2.657	3	3	3.997-4.135	18.224	21.057
Liver.....	100	69.0	4.3	69.0	2	5.975-6.139	6.057	3	2	9.222-9.556	30.116	34.966
Rib roast:												
Separable lean only.....	52	69.8	7.9	69.8	3	.192-.214	.202	3	3	.513-.569	1.795	2.430
Total edible.....	85						.124				.332	
Round, ground.....	100	65.9	12.8	65.9	2		.294	3	2	.586-.697	1.924	3.080
Stew meat:												
Separable lean only.....	82	74.1	3.2	74.1	2	.280-.283	.272	3	2	.665-.720	2.080	3.057
Total edible.....	99						.225				.575	
Tongue, smoked:												
Separable lean only.....	74	65.1	10.5	65.1	2	.493-.546	.520	3	2	.722-.752	2.100	3.004
Total edible.....	91						.423				.596	
Lamb:												
Kidney.....	97	80.2	3.0	80.2	2	3.511-3.771	3.641	5	3	4.535-5.132	23.955	28.232
Liver.....	96	63.4	16.1	63.4	3	4.200-4.778	4.408	3	2	6.756-7.567	19.396	34.629
Leg:												
Separable lean only.....	54	73.8	4.5	73.8	3	.267-.302	.289	3	3	.847-.919	3.382	4.083
Total edible.....	77						.203				.621	
Stew meat:												
Separable lean only.....	43	69.2	9.3	69.2	2	.203-.227	.215	3	2	.618-.749	2.289	3.279
Total edible.....	75						.123				.404	
Pork:												
Liver:												
Fresh.....	95	67.9	2.2	67.9	2	2.979-3.022	3.001	3	2	6.481-7.367	21.832	23.438
Air dried.....		8.6			2	9.367-9.830	9.599	2	1	20.423-21.463	22.914	
Ham, smoked, precooked:												
Separable lean only.....	74	68.2	6.6	68.2	2	.357-.406	.382	3	2	.663-.741	.694	2.754
Total edible.....	97						.291				.529	
Loaf:												
Separable lean only.....	59	71.4	6.2	71.4	2	.419-.455	.437	3	2	.817-.914	2.986	3.813
Total edible.....	78						.331				.646	
Rib:												
Separable lean only.....	57	68.7	10.3	68.7	2	.389-.419	.404	3	2	.855-.903	.885	4.214
Total edible.....	78						.295				.646	
Sausage.....	100	39.1	48.2	39.1	2	.303-.304	.304	4	3	.605-.761	1.120	5.370
Heart, kidney:												
Liver.....	100	72.5	2.3	72.5	2	1.699-1.885	1.792	3	2	2.359-2.385	2.372	
Meat:												
Separable lean only.....	82	75.2	1.6	75.2	2	.479-.561	.520	3	2	4.240-4.919	16.375	17.869
Total edible.....	83						.514				.781	
Veal:												
Heart.....	63	77.4	3.5	77.4	3	.587-.692	.615	3	3	2.746-2.812	2.778	14.545
Kidney.....	87	80.5	2.6	80.5	2	2.145-2.200	2.218	3	2	3.927-4.088	4.011	23.734
Liver.....	92	69.5	2.8	69.5	3	3.038-3.139	3.108	2	2	9.598-9.817	9.708	31.830
Leg:												
Separable lean only.....	78	76.8	.8	76.8	2	.405-.536	.471	3	2	.973-1.067	1.019	4.549
Total edible.....	87						.422				.914	
Stew meat:												
Separable lean only.....	77	73.2	2.4	73.2	3	.409-.465	.438	3	3	1.059-1.138	1.106	4.533
Total edible.....	100						.337				.852	
Meat mixtures:												
Frankfurters.....	100	52.3	31.4	52.3	3	.172-.220	.196	3	2	.408-.439	.425	2.607
Liverwurst.....	96	49.0	30.1	49.0	2	1.717-1.903	1.810	3	2	2.720-2.872	2.780	13.301
POULTRY												
Chicken:												
Heart.....	100				2	1.856-2.032	1.944	2	2	2.547-2.571	2.559	
Liver.....	100	71.5	6.2		2	3.622-4.514	4.068	3	2	6.731-7.653	25.133	32.121

Breast: Muscle only Total edible	December, composite from 5 breasts	47	76.2	1.7	2	2	.651-	.711	.681	2,861	2	2	.820-	.856	.838	3,521	3,792
	77 pct. muscle, 23 pct. skin 2	61	72.7	0.9					.635	2,325					.804	2,945	3,941
Leg: Muscle only Total edible	December, composite of 10 legs	62	76.6	3.7	2	2	.777-	.898	.838	3,581	3	2	1.122-	1.240	1,178	5,034	5,980
	86 pct. muscle, 14 pct. skin 2	72	74.4	0.6					.792	3,094					1,110	4,336	5,842
Skin	January, composite from 3 turkeys	24	60.9	24.5	2	2	.438-	.522	.450	1,228	3	3	.676-	.708	.692	1,770	4,740
	do.																
Turkey:	January, composite from 3 turkeys	100	77.2		2	2	1.110-	1.225	1,171	5,136	2	2	2.629-	2.719	2,674	11,728	4,976
	do.																
Neck:	do.	47	77.0	2.2	1	2	.524-	.580	.552	2,400	1	2	1.003-	1.066	1,035	4,500	4,940
	do.	75	77.5		2	2	.489-	.500	.495	2,200	3	2	.837-	.981	1,909	4,739	5,502
Gizzard:	do.	28	76.2	3.3	2	2	.485-	.589	.537	2,256	3	2	1.030-	1.218	1,128	4,739	5,502
	do.	35	74.7	1.0	4	3	.276-	.339	.309	1,221	3	2	.540-	.643	.591	2,336	2,432
Dark meat, muscle only	do.	75	73.1	5.3					.379	1,409					.748	2,781	3,463
	do.																
Dark and white meat, total edible	85 pct. muscle, 15 pct. skin 2	12	61.2	22.2	3	3	.187-	.245	.216	.557	3	3	.316-	.329	.321	.827	1,934
	(From above 3 turkeys)																
Eggs	June 15, composite of 24 egg whites, Agricultural Research Center	58	88.3		3	3	.074-	.087	.079	.675	1	1			.130	1,111	
	do.																
Whites	June 25, composite of 24 egg whites, Agricultural Research Center	56	88.0		1	1			.076	.633	1	1			.155	1,292	
	do.																
Yolks	July, composite of 24 egg whites, Agricultural Research Center	56	88.9		1	1			.074	.667	1	1			.128	1,153	
	do.																
October, composite of 24 egg whites, Agricultural Research Center	do.	57	88.1		1	1			.087	.731	1	1			.162	1,361	
	do.																
June 15, composite of 24 egg yolks, Agricultural Research Center	do.	31	48.7	20.0	1	1			2,513	4,957	1	1			2,955	5,760	13,251
	do.																
June 25, composite of 24 egg yolks, Agricultural Research Center	do.	31	48.4	30.0	1	1			3,811	7,386	1	1			4,606	8,926	21,324
	do.																
July, composite of 24 egg yolks, Agricultural Research Center	do.	31	48.8	27.8	1	1			3,831	7,482	1	1			4,335	8,467	18,526
	do.																
October, composite of 24 egg yolks, Agricultural Research Center	do.	30	48.6	28.0	1	1			4,248	8,265	1	1			5,042	9,809	21,547
	do.																
Whole	June 15: 65 pct. white, 35 pct. yolk 2	89	74.5	10.1					.935	3,667					1,114	4,369	7,234
	do.																
June 25: 64 pct. white, 36 pct. yolk 2	do.	87	73.9	10.7					1,407	5,391					1,741	6,670	11,305
	do.																
July 64 pct. white, 36 pct. yolk 2	do.	87	74.6	9.9					1,413	5,563					1,627	6,406	10,497
	do.																
October 66 pct. white, 34 pct. yolk 2	do.	87	74.7	9.5					1,502	5,937					1,821	7,198	11,525
	do.																
Whole, dried	Commercial, spray-dried, Missouri, special sample	100	3.8	41.1	3	3	4.746-	6.425	5,527	5,745	2	2	7.280-	7.410	7,345	13,330	
	do.																
NUTS	November, ten 1-lb. packages, California	53	5.3	53.8	3	2	.276-	.285	.281	.297		3	.531-	.639	.578	610	1,413
	do.																
Brazil nuts	December, seven 1-lb. packages, Brazil	41	4.3	69.0	3	3	.098-	.104	.100	.104		3	.215-	.242	.231	.911	863
	do.																
Cashews	December, 5 lb., unroasted, special sample	100	3.1	47.4	3	2	.542-	.553	.550	.563		3	1.118-	1.229	1,162	1,139	2,347
	do.																
Chestnuts	December, 5 lb.	82	52.5	2.1	5	4	.263-	.399	.323	.680	6	5	.417-	.544	.473	.996	1,042
	do.																
Coconuts:	October, 10 coconuts	45	41.5	35.5	2	2	.080-	.088	.084	.144		2	.184-	.208	.193	.330	.839
	do.																
Meat	(From above 10 coconuts)	21	94.7	62.7	2	2	.722-	.804	.763	.807		3	.048-	.054	.050	.943	
	do.																
Milk	November, ten 1-lb. packages	35	5.4										1.116-	1.178	1,146	1,211	3,592
	do.																
Peanuts, roasted:	March, 2 market lots, 15 lb. peanuts		1.7	50.4	1	1			1,317	1,340	2	1	2.293-	2.397	2,345	2,386	4,896
	do.																
Without hulls	March, 10 lb. peanuts	65	2.0	50.8	1	1			1,157	1,181	2	2	1.619-	1.683	1,651	1,685	3,498
	do.																
With hulls	November, 5 lb. peanuts, Virginia	67	1.4	51.4	2	2	1.621-	1.652	1,637	1,660	3	3	2.196-	2.525	2,414	2,448	5,114
	do.																
Hulls	97 pct. nuts, 3 pct. hulls 2	67	1.6	49.9					1,596	1,622					2,353	2,391	4,852
	do.																
Peanuts	(From lot of peanuts from Virginia)	2	6.6		2	2	.254-	.308	.281	.301	2	2	.360-	.400	.380	.407	
	do.																
Walnuts	November, 7 lb.	51	2.5	73.2	3	3	1.048-	1.254	1,147	1,176	4	3	1.681-	1.735	1,707	1,751	7,025
	do.																
VEGETABLES, FRESH	November, five 1-lb. packages, California	46	3.9	68.1	4	4	.542-	.618	.591	.615		4	.885-	1.098	.970	1,009	3,464
	do.																
Asparagus	June, 6 bunches	49	93.2		2	2	.201-	.284	.243	3,574	3	2	.603-	.641	.622	9,147	
	do.																
Beans:	September, 15 lb.	44	70.6		2	2	.219-	.225	.222	.755	3	2	.419-	.470	.452	1,537	
	do.																
Lima	December, 8 lb.	78	90.5		2	2	.034-	.042	.038	.400	3	2	.197-	.209	.202	1,236	
	do.																
Wax	September, 8 lb.	82	91.4		2	2	.067-	.080	.074	.860	3	2	.236-	.263	.250	2,907	
	do.																
Beets:	July, from 10 bunches, New Jersey	19	91.1		2	2	.155-	.200	.178	2,008	3	2	.231-	.283	.257	2,858	
	do.																
Greens only	55 beets	55	88.7		3	3	.101-	.111	.106	.938	5	5	.150-	.216	.196	1,549	
	do.																
Total edible	25 pct. greens, 75 pct. roots 2	74	89.3						1,124	1,159					1,832	1,867	
	do.																
Broccoli	December, 5 bunches, California	49	88.7		3	3	.767-	.778	.774	6,850	4	3	1.064-	1.599	1,287	11,389	
	do.																
Brussels sprouts	October, ten 1-qt. boxes, Long Island	73	85.4		2	2	.328-	.399	.364	2,493	3	2	.639-	.772	.723	4,932	
	do.																
Cabbage:	July, 8 heads, Maryland	74	89.6		3	2	.125-	.164	.148	1,423	3	2	.246-	.256	.252	2,423	
	do.																
Radish	December, 6 heads	80	90.3		2	2	.168-	.175	.172	1,773	3	2	.299-	.373	.324	3,340	
	do.																
Winter	do.	76	90.4		2	2	.141-	.154	.148	1,542	3	2	.235-	.290	.263	2,740	
	do.																

See footnotes at end of table.

TABLE 8.—*Pantothenic acid in edible portion of foods, as determined by microbiological assay with L. plantarum with and without double-enzyme extraction—Con.*

Food	History of sample, including month in which obtained, source, ¹ and composition	Yield of edible portion (raw or processed)	Moisture	Fat (ether extract)	Free pantothenic acid					Total pantothenic acid				
					Sub-samples	Assay periods	Fresh-weight basis		Dry-weight basis, average	Sub-samples	Assay periods	Fresh-weight basis		Dry-weight basis
							Range	Average				Range	Average	
VEGETABLES, FRESH—con.														
Carrots.....	December, 28 carrots, Arizona.....	84	88.2		3	3	0.145-0.157	0.150	1.271	3	2	0.232-0.311	0.272	2.305
Cauliflower.....	December, 5 heads, Arizona.....	34	89.4		2	2	0.666-0.719	0.693	6.538	3	3	1.135-1.223	1.186	11.189
	February, 2 heads.....	47	87.9		2	2	0.533-0.537	0.535	4.421	4	3	0.800-0.888	0.834	6.893
Celery (Pascal):														
Leaves only.....	March, 6 bunches.....	22	93.1		2	2	0.222-0.267	0.245	3.551	3	2	0.359-0.459	0.423	6.130
Stalks only.....	do.....	57	94.4		3	3	0.159-0.175	0.167	2.982	3	2	0.407-0.455	0.429	7.661
Total edible.....	28 pct. leaves, 72 pct. stalks ²	79	94.0					1.90	3.167				0.427	7.117
Corn, sweet yellow.....	August, 60 ears, Maryland.....	25	71.4		2	2	0.294-0.317	0.306	1.070	3	2	0.874-0.914	0.899	3.108
Cucumbers.....	August, 36 cucumbers.....	58	96.3		2	2	0.189-0.201	0.195	5.270	3	2	0.291-0.307	0.296	8.000
Eggplant.....	October, 18 eggplants, Florida.....	81	92.4		3	3	0.089-0.091	0.090	1.184	3	2	0.176-0.220	0.205	2.697
Kale.....	December, 8 lb.....	60	77.6		3	3	0.671-0.767	0.725	3.237	4	3	1.138-1.462	1.288	5.750
Lettuce (iceberg, romaine, and Boston).....	December, 3 heads each of 3 varieties.....	80	94.7		3	3	0.101-0.138	0.119	2.245	3	2	0.337-0.403	0.363	6.849
Mushrooms.....	October, 10 boxes, Pennsylvania.....	75	89.0		3	3	1.843-2.036	1.934	17.882	4	4	2.426-2.973	2.713	24.684
Mustard greens.....	July, 5 lb., Pennsylvania.....	71	90.4		3	2	0.139-0.149	0.143	1.490	4	2	0.234-0.280	0.253	2.635
Onions, green:														
Tops only.....	June, 19 bunches, California.....	30	92.7		3	2	0.038-0.048	0.042	0.575	3	2	0.113-0.134	0.126	1.726
Bulbs only.....	do.....	23	89.4		2	2	0.050-0.053	0.046	0.491	3	2	0.146-0.179	0.168	1.585
Total edible.....	57 pct. tops, 43 pct. bulbs ¹	53	91.3					0.042	0.529				0.144	1.655
Parsnips.....	January, 51 parsnips.....	64	71.2		3	2	0.394-0.463	0.430	1.493	3	2	0.620-0.708	0.676	2.347
Peas.....	June, 1 bu.....	28	78.9		2	2	0.258-0.274	0.266	1.261	4	4	0.795-0.866	0.820	3.886
Peppers, green.....	August, 36 peppers.....	77	94.3		2	2		0.071	1.246	4	4	0.206-0.275	0.237	4.158
Potatoes:														
Idaho, new:														
Peel.....	October, 10 lb. potatoes.....	13	79.8		2	1	0.072-0.090	0.081	0.401	2	1	0.127-0.139	0.133	0.658
Peel.....	do.....	86	75.0		2	2	0.210-0.230	0.220	0.880	3	2	0.253-0.319	0.281	1.124
Total edible.....	13 pct. peel, 87 pct. potato ²	99	75.6					0.202	0.828			0.239-0.287	0.261	1.070
Irish Cobbler, new:														
Peel.....	July, 10 lb. potatoes, Delaware.....	15	75.5		3	3	0.170-0.201	0.182	0.743	3	3	0.232-0.301	0.265	1.082
Peel.....	do.....	84	77.1		3	3	0.331-0.422	0.377	1.646	4	3	0.468-0.597	0.517	2.258
Total edible.....	15 pct. peel, 85 pct. potato ²	99	76.9					0.348	1.506			0.441-0.548	0.478	2.069
Irish Cobbler, 5 months' storage:														
Peel.....	February, special sample, Maine.....	12	79.6		2	2	0.122-0.181	0.152	0.745	3	2	0.256-0.334	0.293	1.436
Peel.....	do.....	85	72.0		2	2	0.250-0.276	0.263	0.939	3	2	0.405-0.430	0.417	1.489
Total edible.....	12 pct. peel, 88 pct. potato ²	97	72.9					0.250	0.923			0.303-0.412	0.402	1.483
Radishes.....	June, 15 bunches.....	54	94.5		3	2	0.055-0.060	0.057	1.036	3	2	0.180-0.192	0.184	3.345
Rutabagas.....	December, 10 rutabagas.....	84	87.7		2	2	0.122-0.127	0.125	1.016	3	2	0.175-0.193	0.181	1.472
Spinach.....	January, 8 lb.....		90.1		2	1	0.127-0.145	0.136	1.374	3	2	0.305-0.319	0.312	3.152
Squash:														
Acorn.....	October, 12 squash.....	66	88.8		3	3	0.292-0.358	0.326	2.911	4	3	0.426-0.569	0.490	4.375
Crownneck.....	July, 18 squash, Maryland.....	92	92.4		3	2	0.107-0.132	0.121	1.592	3	2	0.354-0.421	0.388	5.105
Zucchini.....	September, 6 squash.....	98	94.2		2	2	0.156-0.177	0.167	2.879	3	2	0.322-0.365	0.340	5.862
Sweet potatoes.....	December, 15 lb., North Carolina.....	78	65.1		2	2	0.798-0.833	0.816	2.338	3	2	0.897-0.991	0.935	2.679
Swiss chard.....	July, 5 lb., Maryland.....	72	80.7		2	2	0.080-0.088	0.084	0.816	3	2	0.148-0.190	0.172	1.670
Tomatoes.....	August, 1 pk., Maryland.....	81	94.8		2	2	0.063-0.074	0.069	1.327	3	2	0.277-0.338	0.314	6.038
Turnips:														
Greens only.....	July, 5 lb., Pennsylvania.....	31	91.3		2	2	0.125-0.150	0.138	1.586	3	2	0.363-0.405	0.381	4.379
Roots only.....	January, 27 turnips.....	73	90.3		2	2	0.144-0.148	0.146	1.505	3	2	0.190-0.229	0.204	2.103
VEGETABLES, DRIED														
Beans:														
Cowpeas.....	April, composite of 3 brands.....	97	8.6		2	2	0.763-0.773	0.768	0.840	3	2	1.231-1.250	1.243	1.360
Kidney.....	do.....	99	8.9		4	3	0.218-0.287	0.253	0.278	3	2	0.691-0.692	0.692	0.714
Lima.....	November, composite of 4 brands, California.....	97	10.0		2	2	0.588-0.613	0.601	0.688	3	2	1.257-1.358	1.299	1.443
Navy.....	November, composite of 4 brands.....	99	9.7		3	3	0.399-0.433	0.415	0.460	3	2	1.158-1.271	1.208	1.338
Pinto.....	April, composite of 3 brands.....	98	8.7		5	5	0.300-0.440	0.350	0.383	5	4	0.501-0.841	0.645	0.706
Lentils.....	November, composite of 3 brands.....	100	8.0		2	2	0.904-1.055	0.980	1.065	3	2	1.488-1.511	1.499	1.629

See footnotes at end of table.

TABLE 8.—*Pantothenic acid in edible portion of foods, as determined by microbiological assay with L. plantarum with and without double-enzyme extraction—Con.*

Food	History of sample, including month in which obtained, source, ¹ and composition	Yield of edible portion (raw or processed)	Moisture Percent	Fat (ether extract)	Free pantothenic acid					Total pantothenic acid				
					Sub-samples	Assay periods	Fresh-weight basis		Dry-weight basis, average	Sub-samples	Assay periods	Fresh-weight basis		Dry-weight basis
							Range	Average				Range	Average	
CEREALS, OTHER GRAIN PRODUCTS														
Breads:														
Cracked wheat.....	March, composite of 2 brands.....	100	35.4	---	2	2	0.342-0.362	0.352	0.545	3	3	0.572-0.634	0.607	0.940
Low calorie.....	March, 1 brand.....	100	34.4	---	2	2	288-353	320	488	3	3	571-686	624	951
Rye.....	March, composite of 7 brands.....	100	36.3	---	2	2	272-276	274	430	5	5	446-517	473	743
Sour rye.....	March, composite of 2 brands.....	100	33.4	---	2	2	218-292	255	383	3	3	416-455	436	655
Vienna.....	March, composite of 5 brands.....	100	28.5	---	4	4	157-247	209	284	4	4	342-399	378	514
Wheat.....	March, composite of 6 brands.....	100	34.2	---	2	2	374-376	375	570	3	3	518-538	570	805
White, enriched.....	March, composite of 2 brands.....	100	35.2	---	2	2	543-583	563	869	5	5	714-890	789	1,218
White, enriched.....	December, composite of 9 brands.....	100	34.5	---	2	2	301-302	302	461	3	3	430-444	437	667
Corn:														
Flakes.....	January, composite of 4 brands.....	100	3.4	---	2	2	085-088	087	090	3	3	163-207	190	197
Puffed.....	January, composite of 2 brands.....	100	2.8	---	2	2	117-125	121	124	3	3	275-299	288	296
With soya.....	January, 1 brand.....	100	4.6	---	2	2	503-521	512	537	3	3	860-939	911	955
Oat, puffed.....	January, composite of 2 brands.....	100	2.3	---	2	2	362-448	405	415	3	3	876-991	920	942
Rice, puffed.....	January, composite of 5 brands.....	100	3.8	---	3	3	207-255	224	233	4	4	366-390	378	393
Wheat:														
Bran.....	January, 1 brand.....	100	3.6	---	3	3	1,903-2,008	1,940	2,012	3	3	2,861-2,971	2,903	3,011
Flakes.....	June, composite of 2 brands.....	100	3.1	---	3	3	410-425	416	429	4	4	818-902	870	898
With malted barley, etc.....	November, composite of 2 brands.....	100	4.6	---	2	2	369-417	393	412	3	3	689-747	714	748
Puffed.....	January, composite of 4 brands.....	100	2.0	---	2	2	140-200	173	177	3	3	408-460	432	441
Shredded.....	November, composite of 4 brands.....	100	5.6	---	2	2	433-447	440	466	3	3	674-745	706	748
Breakfast cereals, to-be-cooked:														
Oatmeal.....	January, composite of 3 brands.....	100	5.6	6.4	2	2	490-515	503	533	5	5	1,374-1,488	1,413	1,497
Wheat:														1.598
Farina.....	November, composite of 3 brands, Minnesota.....	100	8.8	---	3	3	256-317	283	310	3	3	576-676	620	680
Whole grain.....	January, composite of 2 brands.....	100	5.4	---	4	4	363-479	421	445	5	5	762-1,036	874	924
Flour:														
Buckwheat.....	November, composite of four 1-lb. packages.....	100	7.3	---	2	2	961-1,049	1,005	1,084	3	3	1,356-1,509	1,450	1,564
Soy.....	January, 1 brand, Michigan.....	100	6.7	22.0	5	3	1,016-1,125	1,081	1,146	6	3	1,618-1,784	1,681	1,783
White, enriched, all purpose.....	November, composite of 3 brands.....	100	8.3	---	3	3	260-311	279	304	3	3	512-579	542	591
Whole wheat.....	December, 1 brand.....	100	9.8	---	4	4	597-749	673	746	3	3	1,015-1,211	1,084	1,202
Grains:														
Barley, pearled.....	April, composite from 3 brands.....	100	8.9	---	4	3	140-165	153	168	3	2	493-517	503	552
Cornmeal:														
White.....	January, composite of 3 brands.....	100	6.1	---	3	3	236-271	250	266	3	3	515-575	546	581
Yellow.....	January, composite of 2 brands.....	100	7.3	---	3	3	259-273	266	287	3	3	611-672	639	689
Rice:														
Brown.....	November, composite of 3 brands.....	99	11.4	---	2	2	620-763	692	781	3	2	953-1,113	1,028	1,160
Precooked.....	September, 2 packages.....	100	9.4	---	3	3	975-994	984	993	3	3	254-312	285	315
White.....	November, composite of 5 brands.....	100	11.7	---	2	2	393-498	446	505	3	3	633-649	640	725
Wild.....	September, 2 packages.....	100	9.0	---	2	2	785-820	793	871	3	2	1,038-1,132	1,085	1,137
Wheat.....	1952 National spring-wheat sample.....	100	7.9	---	2	2	580-607	594	645	2	1	1,073-1,137	1,105	1,200
Grain products:														
Yeast:														
Brewers.....	Standard sample.....	100	8.5	---	100	100	3,694-5,407	4,404	4,813	104	100	9,130-13,648	11,096	12,126
Pastry.....	Special sample.....	100	6.3	---	13	11	689-960	803	857	13	11	8,053-10,000	8,752	9,340
MILK, CREAM, CHEESE														
Milk, fluid:														
Buttermilk.....	June, composite of 4 brands.....	100	90.1	1.8	2	2	188-196	192	1,939	3	2	260-292	281	2,838
Homogenized.....	June, commercial dairy.....	100	87.9	3.0	1	1	212	212	1,752	1	1	294	294	3,231
Pasteurized.....	July, Agricultural Research Center.....	100	87.6	3.5	2	2	239-255	247	1,992	2	2	315-326	321	2,589
	June, commercial dairy.....	100	87.7	4.0	1	1	203	203	1,650	1	1	300	300	2,439
	July, Agricultural Research Center.....	100	87.6	3.7	2	2	3179	259	2,089	2	2	400-404	402	3,242

Raw, whole.....	June, Agricultural Research Center.....	100	85.5	5.7	1	1	232	1,600	1	1	293	2,021	3,330
Skimmed.....	July, Agricultural Research Center.....	100	88.4	3.4	2	2	.251	2,164	2	2	.329	2,836	4,012
	June, commercial dairy added solids.....	100	88.6		1	1	.267	2,567	1	1	.362	3,481	
Milk, evaporated.....	July, Agricultural Research Center.....	100	90.3	.3	2	2	.288	2,969	2	2	.387	3,990	4,117
Milk, nonfat dry solids.....	February, composite of 3 brands.....	100	73.6		3	3	.474	1,818	4	4	.665	2,519	
	November, composite of 3 brands.....	100	4.4		2	2	2,350-2,640	2,610	3	3	3,390-3,496	3,611	
	process, Alabama, Minnesota, New York.....	100	1.0		2	2	2,024-2,927	2,776	3	3	3,084-3,867	3,526	
	June, composite of 4 brands 1953 process, Minnesota, New York, Wisconsin, N.Y. soured.....	100	55.7	43.1	2	2	.172-.189	.181	2	2	.249-.261	.576	2,125
Cream, heavy.....	December, composite of 3 brands, 1951 process, Wisconsin, New York.....	95	33.3	34.5	3	3	.200-.272	.243	3	3	.334-.462	.607	1,258
Cheddar.....	April, composite of 5 brands.....	100	78.1	4.5	3	3	.110-.122	.115	4	4	.241-.316	1,279	1,609
Cottage.....	January, composite of 7 brands.....	100	41.0	26.1	2	2	.209-.291	.250	3	3	.467-.494	.817	1,465
Processed.....													

¹ All products were obtained from the Washington, D. C., market, unless otherwise specified.

² Calculated from data obtained on fat-trim or yield.